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# ON THE EXISTENCE OF MORE THAN FOUR ISOAGGLUTININ GROUPS IN HUMAN BLOOD

PART I.*


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## I. Introduction

The existence of isoagglutinins in human blood has been recognized for a little more than twenty-three years. The earliest mention of this subject which we have found was in communications made by Samuel G. Shattock [1] to the London Pathological Society in February and May 1899, on "Chromocyte clumping in acute pneumonia and certain other diseases, and the significance of the buffy coat in the shed blood."

The credit for this discovery is usually accorded to Landsteiner,[2] but his first reference to isoagglutination in human blood appeared on March 3, 1900, almost a year later. This contribution occupied eight lines and appeared as a foot-note on page 361 of his article on a wholly different theme. It was so brief that we take the liberty of quoting it entire.

"(1) Das Serum gesunder Menschen wirkt nicht nur auf tierische Blutkörperchen agglutinierend, sondern öfters auch auf menschliche, von anderen Individuen stammende. Es bleibt zu entscheiden, ob diese Erscheinung durch ursprüngliche individuelle Verschiedenheiten oder durch die erfolgte Einwirkung von Schädigungen etwa bakterieller Natur bedingt ist. Thatsächlich fand ich das erwähnte Verhalten bei Blut, das von Schwerkranken herrührte, besonders ausgeprägt. Es könnte diese Erscheinung mit dem von Maragliano geschilderten Lösungsvermögen des Serums für Blutkörperchen bei verschiedenen Krankheiten zusammenhängen. (IX. Kongr. f. inn. Med., 1892)."

Following these publications a number of authors wrote upon this subject,[3] but their work was chiefly concerned with the diagnostic value of the reaction in various diseases and the occurrence of isoagglutinins in the bloods of mothers and their new-born children.

Landsteiner's real contribution appeared in 1901 [4] when he showed that isoagglutination was independent of health or disease and that it was not a random reaction but followed certain definite laws. He showed that the blood of twenty-two persons investigated by him fell in most cases ("in den meisten Fällen") into three distinct groups, which he called A, B and C. The serum of members of group A agglutinated the red cells of all members of Group B and no others. The serum of members of Group B agglutinated the red cells of all members of Group A and no others. The serum of Group C agglutinated the red cells of both Group A and Group B, but not those of Group C. To explain these reactions he felt that it was necessary to assume the presence of at least two agglutinins, one present in serum A, the other present in serum B, and both present in serum C.*

The reaction of human bloods according to the classification of Landsteiner is shown in Table I.

TABLE I.

Reactions of Landsteiner's Three Groups

| Serum | Red Cells | | |
|---|---|---|---|
| | A | B | C |
| Group A | 0 | + | 0 |
| Group B | + | 0 | 0 |
| Group C | + | + | 0 |

In the following year (1902), v. Decastello and Sturli [5] further advanced our knowledge of this subject. In a series of 155 persons over the age of six months whose blood they examined, four were encountered who formed an exception to Landsteiner's rule of three isoagglutinin groups. The serum from none of these four persons was capable of agglutinating the red cells of a member of Groups A, B or C, but their red cells were agglutinated by the serum of each of the three groups. Although the authors did not make a definite statement to this effect, a fourth group was thus plainly established.†

The way in which this addition fits into the scheme of Landsteiner is shown in Table II.

TABLE II.

Relations of the Four Blood Groups

| Serum | Red Cells | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Group A (Landsteiner) | 0 | + | 0 | + |
| Group B (Landsteiner) | + | 0 | 0 | + |
| Group C (Landsteiner) | + | + | 0 | + |
| Group D (v. Decastello & Sturli) | 0 | 0 | 0 | 0 |

* "Man kann der üblichen Ausdrucksweise zufolge sagen, dass in diesen Fällen zumindestens zwei verschiedene Arten von Agglutininen vorhanden sind, die einen in A, die anderen in B, beide zusammen in C. Die Körperchen sind für die Agglutinine, die sich in selben Serum befinden, naturgemäss als unempfindlich anzusehen."

† The paragraph in which v. Decastello and Sturli referred to these exceptional bloods is given below.

"Die 4 Ausnahmen von dieser Regel verhielten sich so, dass das Serum auf kein einziges Probeblut einwirkte, also gar kein Agglutinin enthielt, während die Erythrocyten von jedem anderen Serum agglutinirt wurden, also nicht wie bei den übrigen Personen, zumindestens gegen einen Serumtypus wiederstandsfähig waren."

v. Decastello and Sturli [5] also showed that isoagglutination was not a manifestation of disease but occurred in the same way in normal persons. By absorption experiments they showed that there were two agglutinins, one in serum A, the other in serum B, and both together in serum C.

Five years later (1907), Janský [6] reported the existence of four isoagglutinin groups, three of them with characteristics corresponding to those found by Landsteiner and the fourth in all respects like that formed by the four "exceptions" of v. Decastello and Sturli, but he made no reference to the inadvertent contribution of the latter writers. Further comment on the work of Janský will appear in another section of this report.

In the same year (1907), Hektoen [7] classified bloods into three groups stating that he had encountered but few exceptions to Landsteiner's rule. Although he cited the article of v. Decastello and Sturli, he did not mention the fact that they, too, had encountered exceptions. In a table (Table I, page 298) he bracketed with Group III an example of his exceptions which in its behavior corresponds entirely with that of the fourth group constituted by the exceptions of v. Decastello and Sturli.

In 1909 Moss,[8] also overlooking the real nature of the contribution of v. Decastello and Sturli, and unaware of the work of Janský, again worked out the existence of four isoagglutinin groups. While his paper was in the hands of the printer, he came across the work of Janský to whom, in a foot note, he gave due credit for priority.

Moss numbered his four groups in a different order from that employed by Janský, just as the latter used an order different from that previously employed by Landsteiner and by v. Decastello and Sturli. The Moss Group I corresponded to the Janský IV and the Moss Group IV to the Janský I. It has been asserted that the designations Group II and Group III correspond in the two classifications; probably this is correct.‡

As has been mentioned, the importance of the observations of v. Decastello and Sturli had escaped recognition, apparently even by the observers themselves, and the report of Janský lay buried in an obscure journal. General recognition of the existence of four isoagglutination groups, instead of three as proposed by Landsteiner, is to be dated from the publication by Moss. Unless otherwise plainly indicated, the classification used in this paper is the one proposed by him (see Table VI).

During the eleven years following the first observations published by Shattock,[1] a considerable amount of information accumulated concerning isoagglutination. In addition to the separation of human bloods into four groups on the basis of cross-agglutination reactions, it was definitely proven that the phenomenon was independent of disease. From examination of the blood of infants it was shown that the grouping was often not established at birth; that the red cells acquired agglutinogen as a rule before agglutinin appeared in the serum; and that both agglutinin and agglutinogen appeared, if they were ever to appear, before the child reached the age of six months. All observations pointed to the permanency of these characteristics when once developed. Work was done to show that blood grouping was a hereditary characteristic and followed the Mendelian law. Several authors also suggested that the determination of isoagglutinin groups might have an important bearing upon the selection of donors for transfusion, and the suggestion has been sustained by subsequent experience.

The rapidly growing popularity of blood transfusion as a therapeutic measure, aided by the successive simplifications in its technic, has materially altered the status of blood grouping; from an interesting plaything it has become one of the commonest laboratory procedures. As a result of the increased frequency of application, many modifications of the methods of testing have been proposed and a few of them generally accepted. Among the latter there are two which concern us at the moment.

Moss,[9] in 1911, pointed out that it was unnecessary to use the blood of all four groups in determining the grouping of an unknown blood, as this classification might be accomplished quite as well and more easily (1) by matching the unknown corpuscles against the sera of Groups II and III, or (2) by testing the unknown serum against the cells of Groups II and III. Since with either of these methods the result is either positive or negative in each separate test, there are only four possible combinations;

‡ Groups II and III are alike in the behavior of their serum to the red cells of Group I, and to those of Group IV. They are alike also in that the serum of each agglutinates the red cells of the other. If one is given four bloods and is told only that all the four groups are represented, it is easy by cross-agglutination tests to pick out which is Group I and which is Group IV. By the tests, also, it is simple to tell that the other two are Groups II and III, but impossible, without further information, to say which is which. Hitherto, only two methods have been available for deciding this point, (1) the use of a known II or III serum and (2) the determination of relative frequency. So far as I am aware, no one in this country has had samples of Janský's test serum, so that we must rely upon the second method to settle the matter. Moss assigned the number II to the commoner group (40 per cent) and III to the rarer one (7 per cent). Janský specifically mentions the grouping of bloods from only 30 persons; of these 10 are classified as Group II (33 per cent), and 6 as Group III (20 per cent). He also tested these 30 sera against the whole blood of 69 other persons, and from the behavior of the red cells it is possible to work out the group to which they would be assigned in his classification. Thus his tables show that in a total of 99 persons the red cells of 37 behaved as did those of his 10 members of Group II (37.37 per cent) and the red cells of 19 acted as those of his 6 members of Group III (19.19 per cent). This would seem to indicate that the common and uncommon reciprocals are designated by the same numbers in the classification of Moss and Janský, although the percentage of Group III (19.19 per cent) obtained by the latter is much higher than that found by most other workers. Further comment on the work of Janský will be found in a later section of this paper.

the unknown blood is bound to fall into one of the four known groups, (see Table III).

TABLE III.

First Method

| Serum | $x$ Red Cells | | | |
|---|---|---|---|---|
| Group II | + | 0 | + | 0 |
| Group III | + | + | 0 | 0 |
| Assigns $x$ to | Gr. I | Gr. II | Gr. III | Gr. IV |

Second Method

| Serum | Red Cells | | Assigns $x$ to |
|---|---|---|---|
| | Gr. II | Gr. III | |
| $x$ | 0 | 0 | Group I |
| $x$ | 0 | + | Group II |
| $x$ | + | 0 | Group III |
| $x$ | + | + | Group IV |

Minot[10] in a paper published in May 1916, quoted Vincent as authority for the observation that the grouping of an unknown blood may be determined by cross-agglutination tests of cells and serum against the cells and serum of a known member of either Group II or Group III. The following month Brem [11] read a paper before the meeting of the American Medical Association in which he suggested the same method, (see Table IV).

TABLE IV.

First Method

| Serum | Red Cells | Result | | | |
|---|---|---|---|---|---|
| Group II | $x$ | + | 0 | + | 0 |
| $x$ | Group II | 0 | 0 | + | + |
| Assigns $x$ to | | Gr. I | Gr. II | Gr. III | Gr. IV |

Second Method

| Serum | Red Cells | Result | | | |
|---|---|---|---|---|---|
| Group III | $x$ | + | + | 0 | 0 |
| $x$ | Group III | 0 | + | 0 | + |
| Assigns $x$ to | | Gr. I | Gr. II | Gr. III | Gr. IV |

As may readily be seen, in either experiment there are only four possible combinations of results, so that the unknown blood is sure to fall into one of the four known groups.

It is interesting to note that in the thirteen years which have elapsed since the publication of the report of Moss, despite the great number of persons who have been engaged in grouping bloods and the really stupendous number of tests that have been performed, no group has been subtracted from the accepted four and no one has seriously proposed the addition of another;—interesting, but not surprising. The methods in general use are based upon the assumption that exactly four groups exist, neither more nor less, and as long as these methods prevail, any modification of this view would be most unlikely to arise except by chance. It was largely chance that led to the observations which form the basis of this report.

## II. A New Isoagglutinin Group

1. *The discovery of a blood showing unusual agglutination reactions.*

In the study of a case of so-called "sickle cell anæmia" upon which we hope to report at another time, it became desirable to test the patient's cells and serum against serum and cells from normal persons representing each of the four isoagglutinin groups. The grouping of the patient's blood had previously been determined by matching her red cells against fresh sera of Groups II and III. Inasmuch as her cells were agglutinated by Group II serum but not by Group III, she was considered to belong to Group III, (see Table III). We then proceeded to make the contemplated tests in which her cells were subjected to the action of serum from each of the four groups and her serum allowed to act upon red cells from each of the four groups. After recording observations on our preparations for several days, the puzzling results obtained caused us to postpone the experiments originally planned. Before repeating the experiments we decided to group the patient's blood again, and wholly by chance another method was used. This time her serum was

TABLE V.

Results of Cross-Agglutination Tests

| Blood No. | Serum from | RED CELLS | | | | | | | | | | | | | | | | Group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 (I. K.) | | 2 (H.) | | 3 (G. W.) | | 4 (Y. O.) | | 5 (A. G.) | | 6 (J. V.) | | 7 (J. L.) | | 8 (M. G.) | | 9 (C. T.) | | |
| | | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | Agg. | Hæm. | |
| 1 | I. K. | 0 | | + | Yes | + | Yes | + | Yes | + | Yes | 0 | | + | Yes | + | Yes | + | Yes | IV |
| 2 | H. | 0 | | 0 | | 0 | | + | Yes | + | Yes | 0 | | 0 | | + | Yes | + | Yes | II |
| 3 | G. W. | 0 | | 0 | | 0 | | + | Yes | + | Yes | 0 | | 0 | | + | | + | Yes | II |
| 4 | Y. O. | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | I |
| 5 | A. G. | 0 | | + | Yes | + | Yes | + | Yes | 0 | | 0 | | + | Yes | 0 | | 0 | | III |
| 6 | J. V. | 0 | | + | Yes | + | Yes | + | Yes | + | Yes | 0 | | + | Yes | + | Yes | + | Yes | IV |
| 7 | J. L. | 0 | | 0 | | 0 | | + | Yes | + | Yes | 0 | | 0 | | + | | + | | II |
| 8 | M. G. | 0 | | + | | + | Yes | + | | 0 | | 0 | | + | Yes | 0 | | 0 | | III |
| 9 | C. T. | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | ? |

matched against Group II cells, and Group II serum was matched against her cells. Since her serum did not agglutinate the Group II cells and her cells were agglutinated by the Group II serum, she obviously belonged to Group I, and not to Group III (see Table IV). To check our previous error, the patient's cells were again tested with fresh sera from Groups II and III, and again her cells were agglutinated by Group II serum but not by Group III; plainly she was now a member in good standing of Group III (see Table III). Repeating the tests with the other method, her serum failed to agglutinate the cells of Group II, but her cells were promptly agglutinated by Group II serum; according to the rule she must belong to Group I (see Table IV).

A blood giving such contradictory results obviously required further study. Accordingly tests were made in which the patient's red cells and serum were tested against the cells and serum of a number of persons representing the four known blood groups.

With a sterile syringe washed out with salt solution blood was secured by venipuncture. About three-quarters of each specimen of blood was placed in a sterile tube to clot, and the remainder expelled into a centrifuge tube containing 1.5 per cent sodium citrate in 0.85 per cent sodium chloride. These latter tubes were centrifugated until the red cells were sedimented, the supernatant fluid was drawn off and, after three washings with salt solution, a 5 per cent suspension of cells was made in physiological saline. When coagulation was complete in the tubes of whole blood, the clear serum was pipetted off and each specimen of serum tested against each of the red blood cell suspensions, both macroscopically in tubes and microscopically in hanging-drop preparations. For the macroscopic tests 0.5 c.c. of serum was added to a tube containing an equal amount of the 5 per cent. suspension of red cells. The tubes were placed in the water bath at 37° C. for 2 hours and then in the ice-box over night; the readings were made the following morning. The microscopic tests were made in duplicate, using hollow ground slides and the same sera as for the other tests but a more dilute suspension of washed red cells (1 per cent). The slides were examined immediately to see whether the preparations were satisfactory, and again after one hour in the incubator at 37° C. The result of the series of macroscopic tests is shown in Table V.

In Table V hæmolysis is indicated as well as agglutination. In some instances agglutination occurred without hæmolysis, but in no instance did hæmolysis occur without agglutination.

The results of the microscopic tests in duplicate confirmed the results of the macroscopic tests in each instance so far as agglutination was concerned; hæmolysis cannot always be determined with equal assurance in the microscopic tests.

From Table V it can be seen that bloods from the members of the four known groups behaved to each other in the accepted manner, whereas they acted toward the blood of our patient (C.T.) just as they did in the preliminary tests mentioned above. These findings may perhaps be somewhat clearer if we summarize the results of the cross-agglutination tests shown in Table V (see Table VI).

TABLE VI.

Summary of Agglutination Results from Table V.

| Serum | RED CELLS | | | | |
|---|---|---|---|---|---|
| | Gr. I | Gr. II | Gr. III | Gr. IV | C. T. |
| Group I | 0 | 0 | 0 | 0 | 0 |
| Group II | + | 0 | + | 0 | + |
| Group III | + | + | 0 | 0 | 0 |
| Group IV | + | + | + | 0 | + |
| Patient (C. T.) | 0 | 0 | 0 | 0 | 0 |

From Table VI it is seen that the red cells of the patient, C.T., behaved as did those of Group III, in that they were agglutinated by the sera from Groups II and IV but not by sera from Groups I and III. Her serum, on the other hand, did not agglutinate cells from any of the four groups and thus behaved as did the serum of Group I.

Cross-agglutination tests with her blood have been made against the blood of 59 different persons representing the four groups, as follows: Group I, 7; Group II, 26; Group III, 14; Group IV, 12; and results like those shown in Tables V and VI have been obtained at each repetition of the experiment. The patient's blood has been tested in this way on 16 different occasions over a period of three months, as follows: May 19, 23, 26 and 30; June 1, 7, 17, 23 and 27; July 5, 13, 14, 19, 25 and 26; August 12, 1922.

In these respects, therefore, her serum has continued to behave like that of Group I and her red cells like those of Group III.

2. *Resemblance to Group I.*

Since her serum behaves like that of Group I, the question naturally arises as to whether the patient may not actually belong to this group, from which her blood apparently differs only in that her red cells are not agglutinated by Group III serum, although they are agglutinated by serum from Groups II and IV. On this point we have considerable evidence.

Her red cells were never agglutinated by the serum of any of the 14 members of Group III used in our tests, although these sera agglutinated the red cells of 7 known members of Group I at each opportunity. These results cannot be explained on the basis of low agglutinative power of our Group III sera, as titration of some of these sera showed that they were still capable of agglutinating known Group I cells in dilutions of 1 to 24 and in some instances even higher. This point was approached in another way. Group III sera from 5 different persons were tested for agglutinative power in double and triple quantities against the cells of C.T., without producing a positive result. If our patient had belonged to Group I, it

should have been possible to prove this easily by the absorption method, but, as will be seen when we come to the result of these tests, this was not the case. Additional evidence of a different but most definite sort is to be presented in Section III of this paper. We feel justified, therefore, despite the resemblance in the behavior of the patient's serum to that of Group I, in asserting that her blood does not belong to that group.

3. *Resemblance to Group III.*

Since the red cells of C.T. behave like those of Group III, one may properly ask for our evidence that she does not belong to that group. If she were a member of Group III, her serum should be able to agglutinate the red cells of Groups I and II, but this it has failed to do in a large number of trials. That this failure was not due merely to low titre of agglutinins actually present, was shown by the fact that the same results were obtained when double and triple quantities of C.T.'s serum were used. It occurred to us that there might be something in the patient's serum capable of inhibiting agglutination, thus masking the presence of agglutinins. This point was tested in two ways. Successive dilutions of C.T. serum were made from 1 : 2 to 1 : 16, and these various dilutions were used in macroscopic tests against red cells from Group I, Group II, Group III, Group IV and the patient C.T., but no agglutination occurred in any tube. This experiment failed to show the presence of any inhibitory substance which could be so diluted as to permit the demonstration of isoagglutinins for the red cells used. We also tried the effect of the addition of the patient's serum to sera containing isoagglutinins, with negative results. The results are shown in Table VII.

TABLE VII.

Tests for Inhibition of Agglutination by Serum of C. T.

| Tube No. | Serum | Serum | Salt Sol. | R. B. C. 5% Susp. | Agglutination |
|---|---|---|---|---|---|
| 1 | Group II | —— | 1 unit | Group I | + |
| 2 | Group II | C. T. | —— | Group I | + |
| 3 | Group II | —— | 1 unit | Group III | + |
| 4 | Group II | C. T. | —— | Group III | + |
| 5 | Group III | —— | 1 unit | Group I | + |
| 6 | Group III | C. T. | —— | Group I | + |
| 7 | Group III | —— | 1 unit | Group II | + |
| 8 | Group III | C. T. | —— | Group II | + |
| 9 | Group IV | —— | 1 unit | Group I | + |
| 10 | Group IV | C. T. | —— | Group I | + |
| 11 | Group IV | —— | 1 unit | Group II | + |
| 12 | Group IV | C. T. | —— | Group II | + |
| 13 | Group IV | —— | 1 unit | Group III | + |
| 14 | Group IV | C. T. | —— | Group III | + |
| 15 | —— | C. T. | —— | Group I | 0 |
| 16 | —— | C. T. | —— | Group II | 0 |
| 17 | —— | C. T. | —— | Group III | 0 |

Table VII shows that C.T. serum, when added to Group IV serum, did not prevent agglutination of red cells from Groups I, II and III; when added to Group III serum, it did not prevent agglutination of red cells from Groups I and II; when added to Group II serum, it did not prevent agglutination of red cells from Groups I and III. It neither prevented nor increased the agglutinative action; it behaved just as did the salt solution used as a control for each experiment.

We have indicated that isoagglutinins for the red cells of Groups I and II were absent from the serum of C.T., but it may be asked whether this was a permanent or transient deficiency. We do not know, of course; we can say, however, that they have been absent on each occasion when her blood was tested over a period of three months. Additional evidence will be presented in Section III of this report, but even from the observations reported thus far, it seems reasonable to conclude that the patient does not belong to Group III.

4. *Other bloods which behave in a similar way.*

These findings aroused our curiosity concerning the blood groups represented in the family of the patient, C.T. On inquiry we learned that her parents were living and that of 12 children born to them, four survived,—three girls aged 28, 26 and 18 (C.T.), and a boy aged 23. One of the girls was married and had four living children, aged 5, 4, and 3 and a baby of 5 months. We were able to secure blood from all of these and with the specimens performed cross-agglutination tests, including in the series blood from known members of each of the four regular blood groups. The results are shown in Table VIII.

From Table VIII it is seen that the mother (Lucy T.) belongs to Group II, and that the father (Charles T.) and one daughter (Minnette T.) belong to Group III. The blood of the brother (Alexander T.) and that of the married sister (Gracie C.) behave as does that of the patient (C.T.). The union of a Group III (father) and a Group II (mother) has resulted in 12 children; of the four surviving children, one is a typical member of Group III, while the others apparently have red cells like Group III and serum like Group I. The husband of the second sister (Gracie C.) belongs to Group I; of their four children one apparently belongs to Group I (Gustie C.); two to Group II (C.D.C. and L.M.C.); and one (G.V.C.) to the same group as C.T. These tests seem to indicate, therefore, that one child is of the same group as its father; another is of the same group as its mother, and the other two are of the same group as their maternal grandmother. From these observations it is plain that C.T. is not an isolated example of her peculiar blood grouping, since three other persons have been found (Gracie C., Alexander T. and G.V.C.), whose blood reacts in a similar manner when tested by the method of cross-agglutination.

It may also be appropriate to mention here the relationship of blood grouping to another phenomenon occurring in this family, namely, the presence of the so-called "sickle cells" or crescentic red blood corpuscles. The occurrence of these peculiar cells was quite evident in the

TABLE VIII.

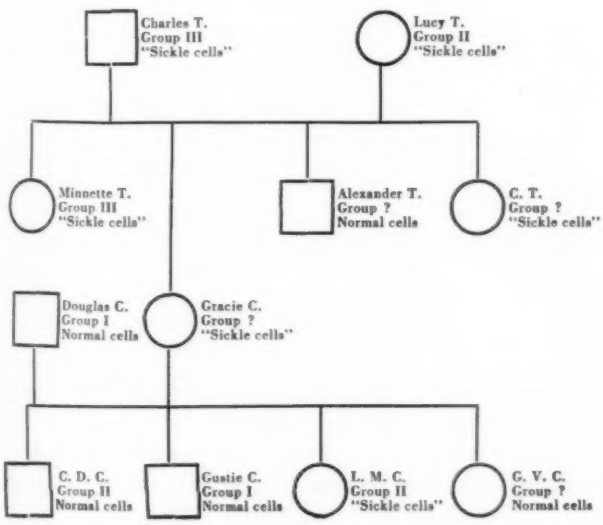
Cross-Agglutination Tests with the Family of C. T.

| Blood No. | Serum from | RED CELLS | | | | | | | | | | | | | | | Blood Group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| 1 | Gr. I (Y. O.) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 2 | Gr. II (H.) | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | II |
| 3 | Gr. III (W. A.) | + | + | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | + | + | + | + | 0 | III |
| 4 | Gr. IV (J. D.) | + | + | + | 0 | + | + | + | + | + | + | + | + | + | + | + | IV |
| 5 | Charles T. | + | + | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | + | + | + | + | 0 | III |
| 6 | Lucy T. | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | II |
| 7 | Minnette T. | + | + | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | + | + | + | + | 0 | III |
| 8 | Alexander T. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| 9 | C. T. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| 10 | Gracie T. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| 11 | Douglas C. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 12 | C. D. C. | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | II |
| 13 | Gustie C. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 14 | L. M. C. | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | II |
| 15 | G. V. C. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |

father (Charles T.); in the mother (Lucy T.); in the two elder sisters (Minnette T. and Gracie C.); in our patient (C.T.) and in one of the children (L.M.C.). They were absent from the blood of the brother (Alexander T.) and three of the children (C.D.C., Gustie C. and G.V.C.). Of the four persons with these unusual agglutination reactions, two exhibited the presence of "sickle cells" (Gracie C. and C.T.) and two did not (Alexander T. and G.V.C.). "Sickle cells" were also found in four other members of the family, two of whom belonged to Group II (Lucy T. and L.M.C.) and the others to Group III (Charles T. and Minnette T.). This may be seen somewhat more clearly in the family tree shown in Table IX.

TABLE IX.

Occurrence of Blood Groups and "Sickle Cells" in the Family of C. T.




From Table IX it is seen that "sickle cells" occurred in some persons belonging to the same group as C.T. and were absent in others, but appeared in four persons belonging to two other groups. This indicates that neither of these unusual manifestations is dependent upon the other.

5. *Absorption experiments.*

Careful absorption experiments were performed, using blood from C.T. and from members of the four recognized groups. The results of these tests are presented in greater detail in Section III, but those which concern us at this time are summarized in Table X.

TABLE X.

Results of Absorption Experiments

| Exp. No. | Serum | Absorbed with R. B. C. | Subsequent agglutination of R.B.C. | | | |
|---|---|---|---|---|---|---|
| | | | Gr. I | Gr. II | Gr. III | C. T. |
| 1 | Group II | Group I | 0 | 0 | 0 | 0 |
| 2 | Group II | Group III | 0 | 0 | 0 | 0 |
| 3 | Group II | C. T. | 0 | 0 | 0 | 0 |
| 4 | Group III | Group I | 0 | 0 | 0 | 0 |
| 5 | Group III | Group II | 0 | 0 | 0 | 0 |
| 6 | Group III | C. T. | + | + | 0 | 0 |
| 7 | Group IV | Group I | 0 | 0 | 0 | 0 |
| 8 | Group IV | Group II | + | 0 | + | + |
| 9 | Group IV | Group III | + | + | 0 | 0 |
| 10 | Group IV | C. T. | + | + | 0 | 0 |

From Table X it is seen that absorption of:

(*a*) Group II serum with r.b.c from Group I, Group III, or C.T., removed all of the agglutinins for the red cells of these three types.

(*b*) Group III serum with r.b.c. from either Group I or Group II, removed all agglutinins for both of these groups.

(*c*) Group III serum with r.b.c. from C.T., did not remove agglutinins for the red cells of Groups I and II. Since Group III serum does not agglutinate the cells of C.T., it was not surprising that absorption with these cells apparently removed no agglutinin.

(*d*) Group IV serum, with r.b.c. from Group I, removed agglutinins for the red cells of Groups I, II, III and C.T.

(*e*) Group IV serum, with r.b.c. from Group II, removed agglutinin for Group II cells, but the serum still agglutinated the cells of Groups I, III and C.T.

(*f*) Group IV serum, with r.b.c. from either Group III or C.T., removed agglutinins for the cells of both types, but the serum still agglutinated the cells of Groups I and II.

It is thus evident that the sera and cells of the four established groups behaved in the traditional way, and that the red cells of C.T. behaved just as did those of Group III.

These results have, of course, a distinct bearing upon the theoretical structure of the four accepted blood groups as well as that of our patient, C.T.

6. *Preliminary classification of the patient's blood.*

TABLE XI.

Blood Formulæ of the Four Groups According to Landsteiner *et al.*

| | Agglutinin in Serum | Agglutinogen in Red Cells |
|---|---|---|
| Group I | *O* | *ab* |
| Group II | *A* | *b* |
| Group III | *B* | *a* |
| Group IV | *AB* | *o* |
| C. T. | *O* | *a* |

To explain the behavior of his three groups, Landsteiner postulated the existence of at least two isoagglutinins. v. Dungern and Hirschfeld [12] assigned the Greek letters $\alpha$ and $\beta$ to designate these agglutinins and ordinary letters to designate the corresponding receptors (agglutinogens) in the red blood cells. When a fourth group was established, it was seen that the theory could be used to explain its structure also, as is seen from Table XI.

TABLE XII.

Blood Formulæ from Table XI. Substituted in Table X.

| Exp. No. | Serum | Absorbed with R. B. C. | Subsequent Agglutination of R. B. C. | | | |
|---|---|---|---|---|---|---|
| | | | *ab* | *b* | *a* | *o* |
| 1 | *A* | *ab* | 0 | 0 | 0 | 0 |
| 2 | *A* | *a* | 0 | 0 | 0 | 0 |
| 3 | *A* | *a* | 0 | 0 | 0 | 0 |
| 4 | *B* | *ab* | 0 | 0 | 0 | 0 |
| 5 | *B* | *b* | 0 | 0 | 0 | 0 |
| 6 | *B* | *a* | + | + | 0 | 0 |
| 7 | *AB* | *ab* | 0 | 0 | 0 | 0 |
| 8 | *AB* | *b* | + | 0 | + | + |
| 9 | *AB* | *a* | + | + | 0 | 0 |
| 10 | *AB* | *a* | + | + | 0 | 0 |

Inasmuch as the serum of C.T. does not agglutinate the cells of any of the four accepted groups and her red cells behave as do those of Group III, we have for present purposes indicated her serum as having no agglutinins and her cells as containing agglutinogen *a*. By substituting these values for serum and cells in Table X it will be seen that the results are intelligible, as shown in Table XII.

It has been mentioned that the existence of two agglutinins and two agglutinogens was used to explain the action of the three blood groups first discovered, and that by adding another combination the theory was found adequate to explain the behavior of the fourth group as well, since which time no further demands have been made upon it. A point which has not attracted attention, however, is that this by no means exhausts the list of combinations of two agglutinins and two agglutinogens. The mathematical possibilities are sixteen, as shown in Table XIII.

TABLE XIII.

Combinations of 2 Agglutinins and 2 Agglutinogens; Mathematical Possibilities

| Comb. No. | Contains | | Comb. No. | Contains | |
|---|---|---|---|---|---|
| | Agglutinin | Agglutinogen | | Agglutinin | Agglutinogen |
| 1 | *O* | *ab* | 9 | *O* | *a* |
| 2 | *A* | *ab* | 10 | *A* | *a* |
| 3 | *B* | *ab* | 11 | *B* | *a* |
| 4 | *AB* | *ab* | 12 | *AB* | *a* |
| 5 | *O* | *b* | 13 | *O* | *o* |
| 6 | *A* | *b* | 14 | *A* | *o* |
| 7 | *B* | *b* | 15 | *B* | *o* |
| 8 | *AB* | *b* | 16 | *AB* | *o* |

TABLE XIV.

Combinations of 2 Agglutinins and 2 Agglutinogens; Biological Possibilities

| No. | Agglutinin | Agglutinogen | Group |
|---|---|---|---|
| 1 | *O* | *ab* | I |
| 6 | *A* | *b* | II |
| 5 | *O* | *b* | |
| 11 | *B* | *a* | III |
| 9 | *O* | *a* | (C. T.) |
| 16 | *AB* | *o* | IV |
| 14 | *A* | *o* | |
| 15 | *B* | *o* | |
| 13 | *O* | *o* | |

After the elimination of those combinations which would indicate the presence of corresponding agglutinins and agglutinogens in the same blood (Numbers 2, 3, 4,

7, 8, 10 and 12), there remain nine which may be regarded as biological possibilities (Numbers 1, 5, 6, 9, 11, 13, 14, 15 and 16). The revised list is shown in Table XIV.

These nine combinations may be expressed either as nine groups or as four main groups with a series of variants or subgroups, none for Group I, one each for Groups II and III and three for Group IV. The inter-reactions of these nine combinations are shown in Table XV.

TABLE XV.

Inter-Reactions of the 9 Combinations Shown in Table XIV.

| Comb. No. | Serum Contains Agglutinin | Red Cells Contain Agglutinogen | | | | | | | | | Serum Reacts as Group |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *ab* | *b* | *b* | *a* | *a* | *o* | *o* | *o* | *o* | |
| | | 1 | 5 | 5 | 9 | 11 | 13 | 14 | 15 | 16 | |
| 1 | *O* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 5 | *O* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 6 | *A* | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | II |
| 9 | *O* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 11 | *B* | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | III |
| 13 | *O* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I |
| 14 | *A* | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | II |
| 15 | *B* | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | III |
| 16 | *AB* | + | + | + | + | + | 0 | 0 | 0 | 0 | IV |
| Cells React as Group | | I | II | II | III | III | IV | IV | IV | IV | |

It may be recalled that at first our patient C.T. was classified alternately as belonging to Group III and to Group I, depending on the type of test employed. In the same way, if encountered, these five additional combinations (Nos. 5, 9, 13, 14, and 15) would be classified differently, according to the method used in determining their status. The resultant effects can be seen in Table XVI.

TABLE XVI.

Classification of the 9 Combinations by Various Methods

| Comb. No. | Formula | | Results of Classification by Different Methods | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 |
| | Agglutinin | Agglutinogen | *x* Cells vs. Ser. II and Ser. III | *x* Serum vs. R. B. C. II and III | *x* Cells and Ser. vs. Gr. II Ser. and Cells | *x* Cells and Ser. vs. Gr. III Ser. and Cells | *x* Serum vs. Cells of Gr. I, II, III, IV | *x* Cells vs. Ser. of Gr. I, II, III, IV | *x* Cells and Ser. vs. Cells and Ser. of Gr. I, II, III, and IV |
| 1 | *O* | *ab* | I | I | I | I | I | I | I |
| 6 | *A* | *b* | II | II | II | II | II | II | II |
| 11 | *B* | *a* | III | III | III | III | III | III | III |
| 16 | *AB* | *o* | IV | IV | IV | IV | IV | IV | IV |
| 5 | *O* | *b* | II | I | II | I | I | II | ? |
| 9 | *O* | *a* | III | I | I | III | I | III | ? |
| 13 | *O* | *o* | IV | I | II | III | I | IV | ? |
| 14 | *A* | *o* | IV | II | II | IV | II | IV | ? |
| 15 | *B* | *o* | IV | III | IV | III | III | IV | ? |

From Table XVI it is apparent that the combinations representing the four accepted groups (Nos. 1, 6, 11 and 16) show no variation when tested by any of these methods, but it is quite different with the other five biological possibilities. As has been mentioned, combination No. 9 (C.T.) was classified at one time as in Group I, at another as in Group III. Thus, combination No. 5 might be regarded as either I or II; No. 14 as either II or IV; No. 15 as either III or IV; and No. 13 as I, II, III or IV. The use of more than one type of test might reveal discrepancies as it did in the case of our patient, C.T. After assigning a blood representing one of these five combinations to one of the four accepted groups by either of the usual methods (see Tables III and IV), cross-matching with the blood of a true member of the group in question might, or might not, call attention to the error. If complete cross-agglutination tests were made with the unknown blood and bloods from real representatives of all four accepted groups, such discrepancies could not escape recognition.

## III. A Sixth Isoagglutinin Group

### 1. *The discovery of another blood with unusual reactions.*

It has been stated that the serum of our patient (C.T.) did not agglutinate the red cells of any of the 26 members of Group II against which it was tested; we therefore assumed that agglutinin *B* was not present in her blood. Inasmuch as her serum also failed to agglutinate the red cells of any of the 14 members of Group III against which it was tested, it was evident that agglutinin *A* was likewise absent. These conclusions were confirmed by the failure of her serum to agglutinate the red cells of any of the 7 members of Group I against which it was tested, thus demonstrating the absence of both agglutinin *A* and *B*. Her blood was not devoid of isoagglutinins, however, as we chanced to find a person with red cells which her serum could agglutinate. Her serum agglutinated the cells of this individual not feebly, not merely once or twice, but decisively and at each one of a great number of tests over a period of two months. The blood of this person (D.J.), therefore, reacted quite differently from that of the other members of the group to which he apparently belonged.

Although this point will be emphasized in another place, it may not be inappropriate to point out that, inasmuch as the serum of C.T. agglutinated the cells of D.J., it contained an agglutinin; as her serum was devoid of agglutinins *A* and *B*, this reaction could only be explained by the presence of an agglutinin different from either *A* or *B*,—a third agglutinin. Agglutination by a third agglutinin could be accomplished only by its union with a third agglutinogen present in the cells of D.J.

### 2. *Resemblance to Group II.*

D.J. was considered a member of Group II and as such his blood behaved in quite the orthodox manner. His serum did not agglutinate the red cells of Groups II or IV but strongly agglutinated the red cells of Groups I and III. His red cells were not agglutinated by the sera

of Groups I and II but were agglutinated by the sera of Groups III and IV. These reactions have been tested repeatedly and the results have been constant. As long as his blood was tested merely by cross-agglutination with the bloods of members of the four accepted groups, there was nothing to suggest that it was unusual in any respect. He seemed a typical member of Group II and as such he would have continued to be classified had it not been for the agglutination of his cells by the serum of C.T. This selective action of her serum served, however, to differentiate his blood from the other members of Group II, against which her blood was tested. We are indebted to the lucky chance of having one unusual blood (C.T.) for the opportunity of finding another of a different sort (D.J.). It may be pointed out that had the blood of C.T. been tested first and only against that of D.J., she would have been considered an ordinary member of Group III and he would have continued to be regarded as belonging to Group II, since their bloods were mutually agglutinative (see Table IV).

3. *Absorption experiments.*

As his blood obviously differed from that of other members of Group II, it seemed possible that the nature of this difference might be demonstrated by means of absorption tests. Experiments of this sort were carried out extensively and repeatedly, according to the following technic. All sera to be absorbed were first tested for their agglutinative titre against each of the different specimens of red cells that were to be subsequently used for absorption. By this means we obtained information as to the approximate quantity of red cells necessary to absorb from each serum all the agglutinin for those red cells. When these relative proportions had been determined, the appropriate quantity of packed, thrice-washed, red cells was added to the necessary amount of serum and the tubes placed in the ice-chest. By this means absorption occurred quite as efficiently as at room or body temperature and the occurrence of isohæmolysis was avoided. The tubes were gently shaken every 25 or 30 minutes and removed from the ice-box at the end of two hours. The tubes were then rapidly centrifugated until all the cells were sedimented, the clear serum pipetted off and the final tests made. In each tube was placed 0.25 or 0.5 c.c. of the absorbed serum and an equal quantity of fresh, washed red cells. The tubes were then placed in the water-bath at 37° C. for two hours, in the ice-box over night, and readings were made on the following morning. The control tube in each series was that in which the absorbed serum was tested against fresh, washed red cells from the same blood as those used for absorption. The result in this tube showed whether the absorption had been complete or not; from the others in the series we learned whether other agglutinins were present after the removal of those originally absorbed. To guard against the effect of individual differences which we had begun to suspect might occur in the bloods of persons apparently belonging to the same group, blood from the same persons was used throughout these absorption experiments. A sample protocol is presented in Table XVII and a summary of the results of various experiments in Table XVIII.

TABLE XVII.

Protocol of Experiment 12

| Tube No. | Serum | Absorbed with R. B. C. | Subsequent Agglutination with Red Cells | | | | | Result |
|---|---|---|---|---|---|---|---|---|
| 56 | Gr. IV (J.D.) | Gr. II (H.) | Gr. I (Y.O.) | — | — | — | — | + |
| 57 | " | " | — | Gr. II (H.) | — | — | — | 0 |
| 58 | " | " | — | — | Gr. II (D.J.) | — | — | + |
| 59 | " | " | — | — | — | Gr. III (L.K.) | — | + |
| 60 | " | " | — | — | — | — | C.T. | + |

Concerning the results summarized in Table XVIII it may be worth while to speak more in detail.

(*a*) It is apparent that red blood cells of the types used for the preliminary absorption were not agglutinated in the subsequent tests; in other words the absorption was complete in each instance.§

TABLE XVIII.

Results of Absorption Experiments

| Exp. No. | Serum | Absorbed with R. B. C. | Subsequent Agglutination of Red Cells | | | | |
|---|---|---|---|---|---|---|---|
| | | | Gr. I (Y.O.) | Gr. II (H.) | Gr. II (D.J.) | Gr. III (L.K.) | C.T. |
| 1 | Gr. II (H.) | Gr. I (Y.O.) | 0 | 0 | 0 | 0 | 0 |
| 2 | Gr. II (H.) | Gr. III(L.K.) | 0 | 0 | 0 | 0 | 0 |
| 3 | Gr. II (H.) | C. T. | 0 | 0 | 0 | 0 | 0 |
| 4 | Gr. II (D.J.) | Gr. I (Y.O.) | 0 | 0 | 0 | 0 | 0 |
| 5 | Gr. II (D.J.) | Gr. III(L.K.) | 0 | 0 | 0 | 0 | 0 |
| 6 | Gr. II (D.J.) | C. T. | 0 | 0 | 0 | 0 | 0 |
| 7 | Gr. III(L.K.) | Gr. I (Y.O.) | 0 | 0 | + | 0 | 0 |
| 8 | Gr. III(L.K.) | Gr. II (H.) | 0 | 0 | + | 0 | 0 |
| 9 | Gr. III(L.K.) | Gr. II (D.J.) | 0 | 0 | 0 | 0 | 0 |
| 10 | Gr. III(L.K.) | C. T. | + | + | + | 0 | 0 |
| 11 | Gr. IV (J.D.) | Gr. I (Y.O.) | 0 | 0 | + | 0 | 0 |
| 12 | Gr. IV (J.D.) | Gr. II (H.) | + | 0 | + | + | + |
| 13 | Gr. IV (J.D.) | Gr. II (D.J.) | + | 0 | 0 | + | + |
| 14 | Gr. IV (J.D.) | Gr. III(L.K.) | + | + | + | 0 | 0 |
| 15 | Gr. IV (J.D.) | C. T. | + | + | + | 0 | 0 |

§ This does not mean that we invariably succeeded; in the beginning we did not; but by study of the agglutinative titre of the various sera, we learned to estimate the quantity of red cells necessary for complete absorption and thus insure satisfactory results.

The actual amount of thrice-washed, packed red cells which we found it necessary to use was proportionally much greater than that recommended by Ottenberg.[13] If packed cells be considered as a 75 per cent suspension, we used approximately 9 volumes of cells to 20 volumes of serum, whereas Ottenberg found that one volume of cells would completely remove the isoagglutinin from 16 volumes of serum. As the absorption in both cases was carried out in the ice-box, the explanation of the difference in proportions required is probably to be found in the time element; our sera were absorbed for 2 hours,—his over night.

(*b*) In no instance was a serum, after absorption, capable of agglutinating cells which it was unable to agglutinate before. Thus Group III serum did not agglutinate the cells of C.T., nor did a serum ever agglutinate cells from its own blood.

(*c*) Groups II (H.) serum when absorbed with the cells of Group I, Group III or C.T., lost all power to agglutinate any cells in the series.

(*d*) Group II (D.J.) serum behaved in the same way as Group II (H.) serum.

(*e*) Group III serum, after absorption with Group I cells, was incapable of agglutinating the cells of Group I, Group II (H.), Group III or C.T., but did agglutinate the cells of Group II (D.J.).

(*f*) Group III serum, after absorption with the cells of group II (H.), behaved in the same way as after absorption with the cells of Group I.

(*g*) Group III serum, after absorption with the cells of Group II (D.J.), lost all power to agglutinate any cells in the series.

(*h*) Group III serum, after absorption with the cells of C.T., lost none of its agglutinative power and behaved just as before absorption.

(*i*) Group IV serum, after absorption with the cells of Group I, no longer agglutinated the cells of Group I, Group II (H.), Group III, or C.T., but did agglutinate the cells of Group II (D.J.).

(*j*) Group IV serum, after absorption with the cells of Group II (H.), lost the power to agglutinate the cells of Group II (H.), but still agglutinated all others in the series.

(*k*) Group IV serum, after absorption with the cells of Group II (D.J.), no longer agglutinated the cells of either Group II (H.) or Group II (D.J.), but agglutinated all others in the series.

(*l*) Group IV serum, after absorption with the cells of Group III, no longer agglutinated the cells of Group III or C.T., but did agglutinate the cells of Group I, Group II (H.), and Group II (D.J.).

(*m*) Group IV serum, after absorption with the cells of C.T., behaved in the same way as after absorption with the cells of Group III.

It is thus seen that the sera of Group II (H.) and Group II (D.J.) behaved alike (Experiments 1 to 6). When deprived of their agglutinin for any of the three types of cells used for absorption, they lost agglutinin for all. This can be explained by assuming the presence in these sera of a single sort of agglutinin (*A*) acting upon the corresponding agglutinogen (*a*) present in the cells of Group I, Group III and C.T.

The red cells of Group II (H.) and Group II (D.J.), on the contrary, did not act alike. Agglutinin for the cells of Group II (H.) was completely removed from Group III serum (Exp. 7 and 8), and from Group IV serum (Exp. 11 and 12), but there was still present in each strong agglutinative power for the cells of Group II (D.J.). This may be explained by assuming the presence of an agglutinogen (*b*) possessed by the red cells of both (H.) and (D.J.), and an additional agglutinogen (*c*) possessed by those of (D.J.) alone. This also necessitates the presence of the corresponding agglutinin (*C*) in the sera of Groups III and IV.

After removal from serum IV of the agglutinin for the cells of Group II (H.), the agglutinin for Group III was still present (Exp. 12); likewise, after removal of the agglutinin for Group III, the agglutinin for Group II (H.) was still present (Exp. 14). Removal of the agglutinin for Group I, however, removed that for both Group II (H.) and Group III. It is also to be recalled that Group II (H.) and Group III are reciprocally agglutinative and both agglutinate the cells of Group I. This can be explained in the accepted manner by assuming the presence of an agglutinogen (*a*) in the cells of Group III corresponding o the agglutinin (*A*) in the serum of Group II (H.); in the serum of Group III an agglutinin (*B*) corresponding to the agglutinogen (*b*) in the cells of Group II (H.); and in the cells of Group I both of the agglutinogens (*a* and *b*) possessed by the cells of Group II (H.) and Group III.

Since Group IV serum agglutinated the cells of Group II (H.) and Group III, this serum must contain the agglutinins (*A* and *B*) corresponding to the agglutinogen (*b*) present in the cells of Group II (H.) and the agglutinogen (*a*) present in the cells of Group III.

The cells of Group III and C.T. behaved alike throughout; when agglutinin was present for one, it was present for the other; absorption with one produced just the same effect as absorption with the other. It is, therefore, fair to assume for the cells of C.T., the same agglutinogen (*a*) as that present in the cells of Group III, and likewise to assume the absence of agglutinogens (*b* and *c*) corresponding to the agglutinins (*B* and *C*) present in the serum of Group III, since her cells are not agglutinated by that serum. It has been shown in Section II that the serum of C.T. does not agglutinate the cells of Group I, Group II (H.) or Group III, and so must be devoid of agglutinin *A* and of agglutinin *B*. Her serum does agglutinate, however, the cells of Group II (D.J.), which can only be due to the presence of another agglutinin (*C*), necessitating the presence of the corresponding agglutinogen (*c*) in the cells of (D.J.). This latter assumption we have also indicated as necessary to explain the result of our absorption tests.

The structure of these bloods as we conceive them to be, is shown in Table XIX.

For the present we might describe the blood of C.T. as belonging to a Group III whose serum was devoid of agglutinin *B*, and that of D.J. as belonging to a Group II whose red cells contained an extra agglutinogen (*c*). In reality each of these two bloods constitutes an addi-

TABLE XIX.

Blood Formulæ of the 6 Persons Shown in Table XVIII.

| Blood | Serum contains Agglutinin | Red Cells contain Agglutinogen |
|---|---|---|
| Gr. I (Y.O.) | *0* | *ab* |
| Gr. II (H.) | *A* | *b* |
| Gr. II (D.J.) | *A* | *bc* |
| Gr. III (L.K.) | *BC* | *a* |
| Gr. IV (J.D.) | *ABC* | *o* |
| C. T. | *C* | *a* |

tional blood group,separate and distinct from the established four. An unsuspected agglutinin has been demonstrated in the serum of the representatives of Groups III and IV used in our absorption experiments. Only the bloods of Group I and Group II (H.) behaved in a manner to accord with the theoretical structure ordinarily assigned to them.

4. *Other bloods which behave in the same way.*

The cleavage effected by the preceding experiments is apparent. The traditional Group II is not a unit, but is used to designate at least two, superficially similar, but actually quite different, types of blood. As has been shown, the serum of each type apparently contains a single agglutinin (*A*), but the red cells of Group II (D.J.) contain two agglutinogens (*b* and *c*), whereas those of Group II (H.) have only one (*b*). We cannot state with absolute assurance which is the commoner of these two types, as it is easy to be misled from a small series. Absorption tests to determine this point have not been carried out extensively. We have been able, however, to test this point more directly. Red blood cells from 142 patients in the wards of the hospital were tested with the serum of C.T., and those from 13 were agglutinated. Cross-agglutination tests were then made with these 13 bloods, the blood of C.T., Group II (H.), Group II (D.J.), and blood from tested representatives of Group I, Group III and Group IV. The results of these cross-agglutination tests are shown in Table XX.

TABLE XX.

Cross-Agglutination Tests with Bloods from Unusual Members of Group II.

| Blood No. | Serum | RED CELLS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 to 19 |
| 1 | Gr. I (G.S.) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | Gr. II (H.) | + | 0 | 0 | + | 0 | + | 0 |
| 3 | Gr. II (D.J.) | + | 0 | 0 | + | 0 | + | 0 |
| 4 | Gr. III (A.H) | + | + | + | 0 | 0 | 0 | + |
| 5 | Gr. IV (S.P.) | + | + | + | + | 0 | + | + |
| 6 | C. T. | 0 | 0 | + | 0 | 0 | 0 | + |
| 7-19 | Each of 13 patients | + | 0 | 0 | + | 0 | + | 0 |

Table XX shows that the bloods of each of these 13 patients reacted to the members of the 4 known groups like members of Group II, but that their behavior with the blood of C.T. was like that of Group II (D.J.), rather than like that of Group II (H.). We were unable to continue this work until 12 days later, by which time all but 4 of the 13 patients had left the hospital. The serum of C.T. again agglutinated the cells of these 4 patients and the cells of Group II (D.J.). After absorption of C.T. serum with the cells of D.J., it agglutinated the cells of none of these 5 persons.

Unfortunately, we did not group all of the 142 bloods used in the experiment, but only those of the 13 agglutinated by the serum of C.T., all of which behaved like Group II. In the total number we might have expected to find about 40 per cent or 56 members of Group II. From this it would seem that the formula *A—b* is commoner than *A—bc*.

NOTE BY THE AUTHORS

As our rather voluminous report has been divided into two parts merely to facilitate publication, comment on the observations presented here is withheld at this time. The second installment containing sundry corroborative evidence, suggestions as to application, summary, conclusions and complete bibliography, is to appear in the next number of this Bulletin.

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# THE BLOOD PRESSURES IN THE UNANESTHETIZED DOG

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During the course of some experiments in which routine determinations were made on a number of dogs with the method described by one of us,[1] it frequently happened that the levels of pressure as indicated by the graphic oscillatory method were surprisingly low for the diastolic pressure. The systolic pressures were within the range of pressures recorded by Dawson [2] and by Wiggers [3] for the anesthetized dog. Dawson's data place the diastolic pressure at about 100 mm., while the systolic pressures found by him in various parts of the arterial tree average approximately 160 mm. The pulse pressure in the dog under ether is, then, about 60 mm. Such pressures have been quite the exception in our experience with the unanesthetized animal. The pressures reported by Wiggers are figures obtained during the comparison of different types of recording devices. They are extremely varied, and it appears probable that such variation was due to conditions incident to the experimental procedure. They differ from Dawson's figures in that several very low diastolic readings occur, but the high pulse pressures agree more closely with our determinations than do those recorded by Dawson.

Data concerning the systolic and diastolic pressures in the unanesthetized dog are very meager. Some scattered observations have been recorded, but they have been obtained with methods which have not been controlled with simultaneous direct determinations.

The method employed by us has previously been found to agree very well with direct determinations made with the maximum-minimum valve during anesthesia, and in such check experiments the diastolic pressure was, as a rule, higher than was found in unanesthetized animals. In order to assure ourselves of the accuracy of our results with various pulse rates and other factors which might enter into the case of the unanesthetized dog, we repeated control experiments without general anesthesia. The results of these experiments were entirely satisfactory, although it must be said that the indirect method did not always check as well with the direct determinations as had been the case under ether. The variation we attribute to the more labile pressures in the experiments without ether and the difficulty in making the indirect determination at the moment that the manometer of the maximum-minimum apparatus has come to rest.

## EXPERIMENTAL

The unanesthetized animals studied were a group of medium-sized dogs, 8 males and 4 females. They form part of a series which one of us (Cash) is using for the study of pressure variations following destruction of renal substance. The observations to be recorded in the present work represent the preliminary or preoperative portion of his series of studies. Two hundred and eighty-five determinations were made on the 12 animals. Each animal was followed over a period of time, and several determinations were made on each day of observation. The same room was used throughout, and precautions were taken to insure quiet while the observations were being made. Sufficient time elapsed before observations to allow for the recovery from exercise. Table I gives a summary of this set of experiments. Some of the individual determinations will be found in the protocols at the end of the paper.

Four control experiments were done under morphine. The femoral artery was isolated without discomfort to the animal by the previous subcutaneous injection of a small amount of 0.5 per cent procaine in the immediate region of the intended operation. After the direct and indirect methods had been compared repeatedly, ether was administered from a cone and general anesthesia was maintained while a second series of comparisons was made. In Table 2 it will be seen that the two methods with one or two exceptions agree very well. The sudden changes of pressure which sometimes occur will account for the discrepancy of 10 mm. found in the second set of determinations of the diastolic pressure in Dog C, under morphine only.

## DISCUSSION

If a simple average is made of the average pressures found in the individual dog, as has been done in Table 1, the systolic level is found to be 165 mm. and the diastolic level, 61 mm. Thus the pulse pressure in the dogs of this series is 104 mm. The extremes show considerable variation from these figures, the greatest variation occurring in the diastolic pressure, where the highest determination is 104 mm. and the lowest, 28 mm. Much of the fluctuation in the individual animal is associated with the emotional reaction, as is also the general level of the pressures found, for it will be noted in the protocols that the reactive animals have consistently higher pressures than the uniformly quiet ones. That the animal may show considerable elevation of pressure due to slight causes is demonstrated by Dog 7, which on May 2nd, after a series of 18 determinations placed the highest systolic reading at 160 mm. and the highest diastolic reading at 58 mm., showed a pressure of 174/80 which was confirmed by a second determination, 168/82. After about half an hour

the pressures were 144/38. The sudden transient rise was due, no doubt, to the clipping of the hair of the leg just before the observations were made. We have had the opportunity to confirm the fact that manipulations such as this will often cause transient elevation of pressure, and have frequently seen the pressure rise because of noises in the laboratory. Particularly is this true of the alert dog who is keenly aware of his surroundings. It is evident from an inspection of the summaries at the end of this paper that the blood pressure of the dog is very labile, even under the conditions of the experiments.

## Conclusions

The systolic blood pressure of the unanesthetized dog at rest is approximately 165 mm. and the diastolic pressure is approximately 60 mm. The pulse pressure under such conditions, then, is about 105 mm. Psychic variations in pressure are common and may be of considerable magnitude.

TABLE I.
Summary of Observations on Unanesthetized Dogs

| Dog | Sex | Weight | Num. of Observ. | Aver. syst. | Aver. diast. | Aver. pulse pr. |
|---|---|---|---|---|---|---|
| | | K | | mm. | mm. | mm. |
| 7 | male | 8.8 | 86 | 134.3 | 38.5 | 95.8 |
| 11 | female | 16.6 | 23 | 152.0 | 47.6 | 104.6 |
| 12 | male | 16.0 | 23 | 160.6 | 59.8 | 100.8 |
| 15 | female | 14.6 | 31 | 142.4 | 54.6 | 87.8 |
| 16 | female | 11.3 | 20 | 165.8 | 58.2 | 107.6 |
| 17 | male | 11.2 | 19 | 165.4 | 62.8 | 102.3 |
| 19 | male | 10.0 | 24 | 171.0 | 65.7 | 105.3 |
| 20 | female | 11.4 | 7 | 178.7 | 71.7 | 107.0 |
| 21 | male | 14.1 | 7 | 164.3 | 64.0 | 100.3 |
| 22 | male | 11.7 | 26 | 184.9 | 67.3 | 115.6 |
| 23 | male | 12.6 | 13 | 189.0 | 68.8 | 120.2 |
| 24 | male | 5.0 | 6 | 174.5 | 74.5 | 100.0 |
| 12 dogs | 8 males, 4 females | | 285 observations | | | |
| Total average pressures | | | | 165.3 | 60.8 | 104.5 |
| Highest average pressures | | | | 189.0 | 74.5 | 120.2 |
| Lowest average pressures | | | | 134.3 | 38.5 | 87.8 |
| Highest individual pressures | | | | 212.0 | 104.0 | 140.0 |
| Lowest individual pressures | | | | 120.0 | 28.0 | 68.0 |

TABLE II.
Control Experiments

| | Preliminary Readings | | | | Comparison of Direct and Indirect Methods | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before morph. | | After morph. | | Morphine only | | | | Morphine-Ether | | | |
| | | | | | Direct | | Indirect | | Direct | | Indirect | |
| Dog | Syst. | Diast. | Syst. | Diast. | Syst. | Diast. | Syst. | Diast. | Syst. | Diast. | Syst. | Diast. |
| A | 148 | 50 | 152 | 50 | 184 | 56 | 182 | 62 | 172 | 77 | 169 | 73 |
| | 146 | 50 | 158 | 52 | 182 | 58 | 180 | 64 | 168 | 70 | 170 | 74 |
| C | 230 | 70 | 215 | 86 | 213 | 76 | 214 | 86 | 172 | 75 | 170 | 80 |
| | 230 | 78 | 210 | 80 | 198 | 72 | 203 | 77 | 178 | 69 | 182 | 72 |
| D | 196 | 76 | | | 186 | 82 | 190 | 84 | 182 | 84 | 188 | 84 |
| | 187 | 78 | | | 180 | 84 | 184 | 90 | | | | |
| F | 140 | 60 | 134 | 63 | 132 | 53 | 130 | 56 | animal died | | | |
| | 144 | 60 | 137 | 61 | 134 | 54 | 127 | 59 | | | | |

*Dog No. 7.*—Male. Wt. 8.8 kilos. Very quiet animal.

| 86 Determinations. | Systolic | Diastolic | Pulse Pressure |
|---|---|---|---|
| Average pressure | 134.3 | 38.5 | 95.8 |
| Maximum pressure | 174 | 82 | 118 |
| Minimum pressure | 120 | 28 | 76 |

*Dog No. 11.*—Female. Wt. 16.6 kilos. Very quiet animal.

| 23 Determinations. | | | |
|---|---|---|---|
| Average pressure | 152.2 | 47.6 | 104.6 |
| Maximum pressure | 164 | 64.0 | 124.0 |
| Minimum pressure | 140 | 36.0 | 90.0 |

*Dog No. 12.*—Male. Wt. 16.0 kilos. Very quiet animal.

| 23 Determinations. | | | |
|---|---|---|---|
| Average pressure | 160.6 | 59.8 | 100.8 |
| Maximum pressure | 176.0 | 74.0 | 122.0 |
| Minimum pressure | 140.0 | 49.0 | 80.0 |

*Dog No. 15.*—Female. Wt. 14.6 kilos. Very old—Apathetic animal in advanced stage of pregnancy.

| 31 Determinations. | | | |
|---|---|---|---|
| Average pressure | 142.4 | 54.6 | 87.8 |
| Maximum pressure | 160 | 66.0 | 104.0 |
| Minimum pressure | 120 | 42.0 | 68.0 |

*Dog No. 17.*—Male. Wt. 11.2 kilos. Very young dog—never very quiet.

| 19 Determinations. | | | |
|---|---|---|---|
| Average pressure | 165.4 | 62.8 | 102.3 |
| Maximum pressure | 181 | 78.0 | 116.0 |
| Minimum pressure | 155 | 52.0 | 89.0 |

*Dog No. 19.*—Male. Wt. 10 kilos. Very excitable animal.

| 24 Determinations. | | | |
|---|---|---|---|
| Average pressure | 171 | 65.7 | 105.3 |
| Maximum pressure | 183 | 82.0 | 121.0 |
| Minimum pressure | 162 | 58.0 | 91.0 |

*Dog No. 20.*—Female. Wt. 11.4 kilos. Very reactive—never quiet on table.

| 7 Determinations. | | | |
|---|---|---|---|
| Average pressure | 178.7 | 71.7 | 107 |
| Maximum pressure | 200 | 80 | 120 |
| Minimum pressure | 160 | 61 | 98 |

*Dog No. 21.*—Male. Wt. 14.1 kilos. Very quiet animal.

| 7 Determinations. | | | |
|---|---|---|---|
| Average pressure | 164.3 | 64 | 100.3 |
| Maximum pressure | 184 | 68 | 117 |
| Minimum pressure | 152 | 59 | 88 |

*Dog No. 22.*—Male. Wt. 11.7 kilos. Reactive at first, but became quieter.

| 26 Readings. | | | |
|---|---|---|---|
| Average pressure | 184.9 | 67.3 | 115.6 |
| Maximum pressure | 212 | 82 | 140 |
| Minimum pressure | 167 | 60 | 98 |

*Dog No. 23.*—Male. Wt. 12.6 kilos. Quiet animal.

| 13 Determinations. | | | |
|---|---|---|---|
| Average pressure | 189 | 68.8 | 120.2 |
| Maximum pressure | 212 | 104 | 137 |
| Minimum pressure | 174 | 50 | 83 |

*Dog No. 24.*—Male. Wt. 5.0 kilos. Very reactive animal.

| 6 Observations. | | | |
|---|---|---|---|
| Average pressure | 174.5 | 74.5 | 100 |
| Maximum pressure | 182.0 | 83.0 | 112 |
| Minimum pressure | 164.0 | 70.0 | 89 |

*Dog No. 16.*—Female. Wt. 11.3 kilos. Reactive animal, but quiet during observations.

Complete protocol.

| Date | Systolic | Diastolic | Pulse Pressure |
|---|---|---|---|
| July 1, 1922 | 147 | 50 | 97 |
| | 158 | 47 | 111 |
| July 5, 1922 | 153 | 54 | 99 |
| | 156 | 56 | 100 |
| | 148 | 56 | 92 |
| July 21, 1922 | 180 | 60 | 120 |
| | 170 | 66 | 104 |
| | 180 | 55 | 125 |
| July 22, 1922 | 172 | 62 | 110 |
| | 176 | 58 | 118 |
| | 172 | 60 | 112 |
| | 176 | 56 | 120 |
| | 170 | 60 | 110 |
| | 178 | 60 | 118 |
| | 160 | 58 | 102 |
| | 160 | 60 | 100 |
| | 162 | 60 | 102 |
| | 164 | 61 | 103 |
| | 165 | 64 | 102 |
| | 168 | 62 | 106 |
| 20 Determinations. | | | |
| Average | 165.8 | 58.25 | 107.55 |
| Maximum | 180.0 | 66.0 | 125.0 |
| Minimum | 147.0 | 47.0 | 92.0 |

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# ISOAGGLUTINATION IN NEW-BORN INFANTS AND THEIR MOTHERS

## A POSSIBLE RELATIONSHIP BETWEEN INTERAGGLUTINATION AND THE TOXEMIAS OF PREGNANCY

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### INTRODUCTION

This paper deals with isoagglutination in new-born infants and their mothers with special reference to the etiology of eclampsia and preeclamptic toxemia. Interest in the problem was aroused by a consideration of the observation first made by Flexner [1,2] and since confirmed by others, that the hyaline thrombi found in the areas of necrosis in the liver of women dying of puerperal eclampsia are composed of agglutinated red blood cells. Since such thrombi and the periportal liver necroses are so characteristic of the pathology of typical eclampsia,[3,4,5,6] it was assumed that any information regarding the origin of the agglutinated red blood cells composing the thrombi might be of value in the etiological study of this peculiar form of toxemia.

Various possible explanations for the appearance of agglutinated red cells in the maternal blood stream during pregnancy suggest themselves. Among these is the possibility of isoagglutination between maternal and fœtal blood in the event:—(1) that the fœtal blood show the phenomena of isoagglutination; (2) that the blood grouping of the fœtus differ from that of the mother; and (3) that the placental barrier between the two circulations be broken down at some point, allowing fœtal blood to gain access to the maternal circulation. The work recorded here is the result of an attempt to test in an indirect way such a possibility by a study of the isoagglutination reactions in new-born infants and their mothers.

Since this work was begun, a number of papers dealing with isoagglutination in new-born infants and their mothers have appeared in the literature, but no large series of normal cases has as yet been recorded, and, so far as I am aware, none has made a special feature of the toxemias of pregnancy in this connection. Reference to the recent communications, as well as to the older literature, shows that isoagglutination characteristics usually are only partially or not at all developed at the time of birth. Happ [7] in his recent work on the appearance of isoagglutinins in children found, in fact, that the blood grouping was not completely established in some cases until the end of the second year of life, although in a small percentage of cases it was found to be partially established at the time of birth or shortly thereafter.

Very soon after the phenomenon of isoagglutination was first described for adult human blood,[8,9] the blood of new-born infants was tested by Halban,[10] who concluded that their erythrocytes are less frequently agglutinable than those of the adult, and that the serum of blood from the umbilical cord does not so frequently contain agglutinins as does the adult blood. He, pointed out, therefore, that such substances as agglutinins do not necessarily pass freely through the placenta, for the reason that the mother's blood may contain agglutinins which are not to be found in detectable concentration in the serum of her fœtus. Langer [11] reported similar results in a series of eleven cases, but found that the serum of three new-born infants agglutinated the red blood cells of their mothers. Von Decastello and Sturli [12] also concluded from the study of seven new-born infants that they may belong to other isoagglutination groups than their mothers, but that isoagglutination reactions may in some cases be entirely absent from the blood of the former.

Disregarding the previous work on the subject, Cherry

and Langrock [13] compared the isoagglutination reactions of new-born infants with those of their mothers in thirty-four normal cases, with the view of determining whether it is safe to employ the mother's blood for transfusing her own infant without first testing the two bloods against each other. As no tendency to interagglutination was found in any of their cases by the method used, they decided that such transfusions are safe.

Although accepted by many, this conclusion is erroneous, as will be seen from the data presented here as well as from the results just quoted.

Much has been written recently [14-22] about the clinical features of blood transfusion reactions. It is now a widely recognized fact that severe toxic reactions may occur in man, as well as in the lower animals, when unsuitable blood or hemagglutinative and hemolytic serum is transfused from one individual to another. Such reactions occur whether the transfused blood is from an individual of another species, or from an individual of the same species, but of another blood group. Although the reaction usually tends to be more severe when the blood of a foreign species is transfused than when the species is the same but the blood group is dissimilar, the reactions appear in the two instances to be essentially of the same kind. Minor reactions occasionally also occur in man even when the blood grouping has been found by most careful testing to be identical for donor and recipient.

It is noteworthy that these reactions to intravascular agglutination and hemolysis frequently suggest in certain respects the signs and symptoms observed in the toxemias of pregnancy. They consist of any or all of the following clinical phenomena: rigors, fever, nausea and vomiting, embolic and thrombotic phenomena, jaundice, hemoglobinuria, oliguria, albuminuria, œdema, urticaria, pruritus, severe headache, dizziness, blurring of vision, epigastric and lumbar pains, a sensation of constriction about the chest, increase in blood pressure, convulsions, acute anaphylactic shock, coma or finally death. Kimpton [17] describes the mode of death in two of his fatal cases following the use of incompatible human blood for transfusion as being anaphylactic in type. Others have observed similar reactions with death due directly to intravascular agglutination and hemolysis. Even the so-called minor reactions, which occasionally follow transfusions in spite of previous blood matching, frequently assume the character of mild anaphylactic phenomena, especially after repeated injections of blood, as has been observed in cases of pernicious anemia,[14,15,20] and in dogs previously immunized with the blood of other dogs.[23]

Although there is no conclusive evidence as yet that the proteins of individuals of one human blood group act precisely as foreign proteins when introduced parenterally into the bodies of individuals of other groups, the type of reaction often strongly suggests such an explanation. Some reactions resemble serum sickness and others anaphylaxis. It has been shown in the operation of skin grafting also that skin transplants from persons of the same blood group as the patient are much more likely to "take" than those from persons of another group, which affords additional evidence of a fundamental dissimilarity in the body proteins of persons with different blood groups.

Although embolic and thrombotic phenomena are frequently mentioned in the literature, our information regarding the pathological tissue changes produced by transfusion of incompatible blood in man is scanty. Animal experimentation, however, has resulted in some very interesting observations, both clinical and pathological. Hasse [24] (1874), for instance, in studying the effects of transfusing blood of antagonistic species, early pointed out that the recipient suffers from rigors, fever, hematuria and finally suppression of the urine. Ponfick [25] (1875), observed that when an animal was transfused with the blood of another species hemoglobinuria occurred and minute emboli and small lesions were found in the kidneys. Hayem [26,27] later described embolic phenomena with formation of thrombi and infarcts in various organs, following such transfusion.

Flexner,[1,2] and later Pearce [28,29,30] and others, made the observation that agglutinated red blood cells from various sources may form hyaline thrombi in the capillaries and lead to focal necroses, chiefly in the liver, but occasionally in the kidneys and other organs as well. Whether the red blood cells are those of the animal itself, agglutinated by hemagglutinative serum introduced into the blood stream, or agglutinated red blood cells from other sources, the effects are essentially the same, namely, the production in the liver of hyaline capillary thrombi and focal necroses, often of the hemorrhagic type. The kidneys frequently show degenerative changes in the convoluted tubules.

Most pertinent to the present thesis was the work of Pearce who showed that hemagglutinative serum, given intravenously, causes such changes within 24 to 48 hours after injection. If, however, the examination was made before the expiration of the time required for the production of necrosis, the organs showed intense engorgement only. The focal necroses found were characteristically located in the peripheral portion of the liver lobules, although the central portion also was occasionally involved. The altered areas, frequently hemorrhagic, were found at times to surround the portal vessels almost entirely and were most pronounced near the surface of the liver. The reason assigned for the peculiar distribution of the lesions is that the blood flows more slowly in these situations, where the capillary bed is narrower than in other portions of the liver lobule. By special means it was demonstrated that the thrombus formation precedes the production of necrosis and that the cause of

the latter is largely mechanical, and not necessarily toxic as is usually assumed.

The lesions thus described resemble very closely those occurring in eclampsia, and Flexner and others state that death in rabbits transfused with dog's blood is frequently preceded by convulsions.

Murray [31] has likewise directed attention to the similarity between the liver lesions in eclampsia and those described by Pearce in animals following intravascular hemagglutination and hemolysis. In searching for a hemotoxic substance in eclampsia, this author examined for isohemolysins the blood serum of ten infants of normal mothers, as well as of one infant whose mother had eclampsia, but obtained entirely negative results.

In the present investigation isoagglutination alone has been studied and somewhat different results have been obtained. It may be said here, however, that the results briefly reported in this preliminary communication are only suggestive and it is not claimed that the evidence is as yet sufficient to establish the etiology of eclampsia or pre-eclamptic toxemia on this basis.

### METHODS EMPLOYED

The plan adopted for this investigation was to match the blood of new-born infants against that of their mothers and to determine so far as possible the blood group to which each belonged. Both normal and toxemic cases were included in the series.

Samples of blood were collected for this purpose by the internes on the ward, from the severed umbilical cord of the child, just after delivery and from an arm vein of the mother at the same time, and were placed immediately in the refrigerator. Two samples were obtained from both the mother and from the child; in each instance one was collected in a clean, dry test-tube for serum, the other in a relatively large amount of 1.5 per cent sodium citrate solution for red blood cell suspensions. All agglutination tests were carried out on the same day with the fresh blood samples, as indicated in the accompanying schema:

SCHEMA OF ISOAGGLUTINATION STUDY

| Slide No. | Serum of | Red Blood Cells of | RESULT | | | | |
|---|---|---|---|---|---|---|---|
| | | | Immediate | 30 min. | 1 hr. | 3 hr. | 24 hr. |
| 1 | child | mother | | | | | |
| 2 | mother | child | | | | | |
| 3 | Group II | mother | | | | | |
| 4 | " III | mother | | | | | |
| 5 | " II | child | | | | | |
| 6 | " III | child | | | | | |
| 7 | child | GroupIII | | | | | |
| 8 | mother | " III | | | | | |
| 9 | child | " II | | | | | |
| 10 | mother | " II | | | | | |
| 11 | 0.85% NaCl | " III | | | | | |
| 12 | " " | " II | | | | | |
| 13 | " " | mother | | | | | |
| 14 | " " | child | | | | | |

Unfortunately, time did not permit careful study of isohemolysis in most of these cases, but this should be included in future studies, because isohemolytic phenomena may possibly appear in some cases before isoagglutination.

All agglutination tests were done by the microscopic hang-drop method as follows: A small drop of a 3 per cent suspension of red blood cells, washed three times in physiological saline, was placed near the center of a specially cleaned coverslip with a drop of clear serum of similar size and the two drops were thoroughly mixed by means of a clean glass rod used for this single preparation only. The slip was then inverted over a hollow-ground glass slide, sealed with liquid petrolatum and examined immediately under the low power objective to make certain that the mixing had been complete and the distribution of cells was uniform. The preparation was then placed in the incubator at 37° C. and examined at intervals of one-half, one, three, and twenty-four hours. All preparations were set up in duplicate and saline solution controls were employed throughout. When any doubt as to the result arose, the test was repeated with fresh blood samples. Most of the positive tests were very definite and were readable at the end of one-half hour, but occasionally three or more hours were necessary for the completion of the reaction. As will be seen from the above schema, the data obtained were sufficient to reveal in all cases the blood group to which the mother belonged and that of the child so far as it was established, except for the rare instances in which the use of Group I red blood cells may have shown agglutinins in the child's serum for such cells only.

The data called for in the above outline were obtained from 180 obstetrical patients delivered in the Johns Hopkins Hospital, all but a few of whom were primiparæ. The clinical observations were made entirely by the members of the obstetrical staff, and it was not until after the completion of the agglutination studies that their records were consulted and the relevant data abstracted for purposes of correlation.

The data obtained from each history were listed under the following heads: (1) Clinical diagnosis; (2) Race; (3) Age; (4) Number of pregnancy; (5) Condition of child; (6) Condition of placenta; (7) Wassermann reaction of mother and child; (8) Representative or last blood pressure reading recorded before onset of labor and also after delivery; (9) œdema or urticaria; (10) Albuminuria, oliguria or hemoglobinuria; (11) Jaundice; (12) Severe headache, dizziness or blurring of vision; (13) Nausea and vomiting; (14) Epigastric pain, sensation of constriction about the chest, rigors, or convulsions; and (15) Miscellaneous. The two sets of data, namely, the results of the isoagglutination tests and the clinical observations, were then placed together in order that any possible correlation might be made more evident.

## Results

The results of our investigation are brought together in the following tables and the distribution of the toxemic cases is summarized in the graphic charts.

As regards isoagglutination reactions, the 180 cases studied were distributed as follows: In 84 cases the child's blood showed no evidence whatever of isoagglutination. In 52 cases the child's blood group was partially established and appeared to be the same as that of the mother, while in the remaining 44 cases, or 24.5 per cent of the total, the child's group was at least partially established and was incompatible with that of the mother.

TABLE I.

To show extent to which Isoagglutination Characteristics were present in the Blood of New-born Infants

| Child's Blood | Serum Contained Agglutinins for | | | | | | Red Blood Cells were Agglutinated by | | | | | | Blood Group fully established |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No R. B. C. | R. B. C. of at least 1 Group | R. B. C. of Group II only | R. B. C. of Group III only | R. B. C. of Groups II and III | R. B. C. of Mother | No Serum | At least one serum | Group II serum | Group III serum | Both Groups II and III serum | Mother's serum | |
| No. of Cases | 127 | 53 | 14 | 21 | 18 | 5 | 88 | 92 | 30 | 39 | 12 | 42 | 20 |

Table I shows the extent to which agglutinins appear in the serum and receptors on the red blood cells of the new-born infants. It is seen at once that the receptors are present at birth far more commonly than the agglutinins. It is seen also that the blood grouping was apparently fully established in but 20 of the 180 cases, or approximately 11 per cent. Whereas the mother's red blood cells were agglutinated by the child's serum in but five cases, the child's red blood cells were agglutinated by the mother's serum in 42 cases. This shows clearly that isoagglutinins are not freely transmitted through the placenta.

TABLE II.

Distribution of the Mothers among the Four Blood Groups. A comparison with the results reported by others for all adults.

| BLOOD Group (Moss) | Percent in each Blood Group | | | Moss | Happ | Bernheim | Von Dungern and Hirschfeld | Miriam Ohmstead |
|---|---|---|---|---|---|---|---|---|
| | White | Colored | Both races combined | | | | | |
| I | 4 | 0 | 1 | 10 | 3 | 2 | 5.7 | 2 |
| II | 39 | 24 | 28 | 40 | 35 | 40 | 47 | 39 |
| III | 14 | 27 | 23 | 7 | 12 | 15 | 11 | 13 |
| IV | 43 | 49 | 48 | 43 | 50 | 43 | 36 | 46 |

Table II shows the distribution into the four blood groups (Moss classification [32]) of the white and colored mothers separately and mixed, as compared with the results obtained by other observers for adults. Group III occurs in the "both races combined" column in a much higher percentage than is usually found. This is due to the unusual distribution among the colored patients in which Groups II and III were almost equal, while none of the 126 colored mothers belonged to Group I. The distribution among the white patients corresponds roughly with that found by other observers for adults.

Tables III and IV and the accompanying charts summarize particularly the data as regards the cases of toxemia. Although several cases of nephritic toxemia were necessarily included in the series, the chief interest centers about the pre-eclamptic and eclamptic type of toxemia, which makes its appearance in the latter fourth of pregnancy and is characterized by the following clinical features: arterial hypertension, œdema, albuminuria, hemoglobinuria, oliguria, jaundice, severe headache, nausea and vomiting, dizziness, visual disturbances, epigastric pain, a sensation of constriction about the chest, convulsions or coma, and usually by certain metabolic changes. For the present study it has been assumed that definite increase in blood pressure during the latter part of the second half of pregnancy with normal pressure in the early months and again after delivery affords the most reliable guide in the diagnosis of mild toxemia, especially if associated with any of the other symptoms mentioned.

Since the ages of the patients included in this series range between 15 and 26 years, most of them being between 18 and 23 years, the blood pressure values found by Alvarez [33] and others [34] for normal women of this age have been taken as the normal standard. From his careful study of 5,807 normal university women and 2,930 normal men, nearly all between the ages of 18 and 24 years, Alvarez concluded that the blood pressure is much more uniform in young women than in young men, and that the normal systolic pressure for the former ranges between 110 and 120 mm. of mercury. He looked upon any pressure above 127 mm. as abnormal, and his figures agree approximately with those obtained by other observers.

Consequently a safe margin has doubtless been left in the present work, in assuming a systolic pressure above 140 mm. to be a sign of toxemia in our young pregnant women where other factors could reasonably be ruled out. Additional evidence that these are true cases of toxemia is found in the fact that a partial gap exists between these and the normal cases so far as blood pressure level is concerned; for, whereas there were but 10 cases in the present series with systolic pressures ranging between 130 and 140 mm., all but two being below 135, there were 19 others with pressures between 140 and 150 mm. According to the law of probability, this is contrary to what would be

TABLE III.

CASES OF DEFINITE TOXEMIA

| Patient's Case No. | Clinical Diagnosis | CLINICAL SIGNS AND SYMPTOMS | | | | | | | Interagglutination | Blood Group | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Blood Pressure | | Œdema | Albuminuria | Headache | Convulsive Phenomena | Nausea and Vomiting | | | | |
| | | Syst. | Diast. | | | | | | | M. | C. | |
| 11166 | Pre-eclamptic Toxemia | 165 | 110 | Slight | 0 | 0 | 0 | 0 | + | 4 | 2 | |
| 11127 | do. | 155 | 95 | Moderate | Trace | + | 0 | 0 | + | 4 | 2 | |
| 11113 | Post-partum Eclampsia | 158 | 112 | 0 | After convulsion | 0 | Post-partum | 0 | + | 4 | 0 ? | B. P. normal before delivery |
| 11114 | Pre-eclamptic Toxemia | 180 | 110 | 0 | Big trace | + | 0 | 0 | 0 | 4 | 0 | |
| 11999 | do. | 160 | 110 | Moderate | Trace | + | 0 | 0 | + | 4 | 3 | |
| 10788 | do. | 165 | 115 | Marked | 0 | + | 0 | + | + | 4 | 2 | |
| 10819 | do. | 180 | 125 | " | Trace | + | 0 | + | + | 3 | 1/1 | Double ovum twins |
| 10730 | with cardiac dis. | 140 | 85 | Mod. throughout pregnancy | Big Trace | + | 0 | 0 | + | 2 | 0 | |
| 10729 | Eclampsia Typical | 180 | 95 | Moderate | Trace | + | Marked | + | + | 4 | 3 | |
| 10741 | Pre-eclamptic Toxemia | 140 | 110 | Slight | Big Trace | + | 0 | 0 | 0 | 4 | 0 | |
| 10590 | do. ? Nephritic Tox. ? | 170 | 90 | Marked | Big trace | 0 | 0 | 0 | 0 | 2 | 0 | B. P. still high 12 days post partum |
| 10601 | Pre-eclamptic Toxemia | 180 | 130 | " | 0 | + | Feeling of constriction about chest | + | + | 4 | 3 | Blurred vision |
| 10868 | do. | 160 | 90 | " | Trace | + | 0 | + | + | 2 | 3 | |
| 10911 | do. | 175 | 125 | Moderate | Trace | + | 0 | + | + | 2 | 1 | Blurred vision |
| 10909 | Nephritic Toxemia, acute | 148 | 105 | Moderate | Marked | + | 0 | 0 | 0 | 4 | 0 | |
| 10615 | Pre-eclamptic Toxemia | 196 | 120 | Marked | Big trace | + | Epigastric pains | 0 | + | 3 | 1 | |

NOTE: 0 = no. + = yes.

expected, if all were normal, because the mode of the normal was around 115 and the average 120 mm.

In Table III are grouped the cases of outspoken toxemia, distributed as follows: 12 diagnosed clinically as pre-eclamptic toxemia, one typical antepartum eclampsia, one postpartum eclampsia, one nephritic toxemia, and one as doubtful. It will be seen from the table that in 9, or 75 per cent, of the 12 cases of pre-eclamptic toxemia the mother's serum agglutinated her child's red blood corpuscles; while in the remaining three cases isoagglutination reactions were entirely absent from the infant's blood or present in concentrations too weak for detection by the

TABLE IV.

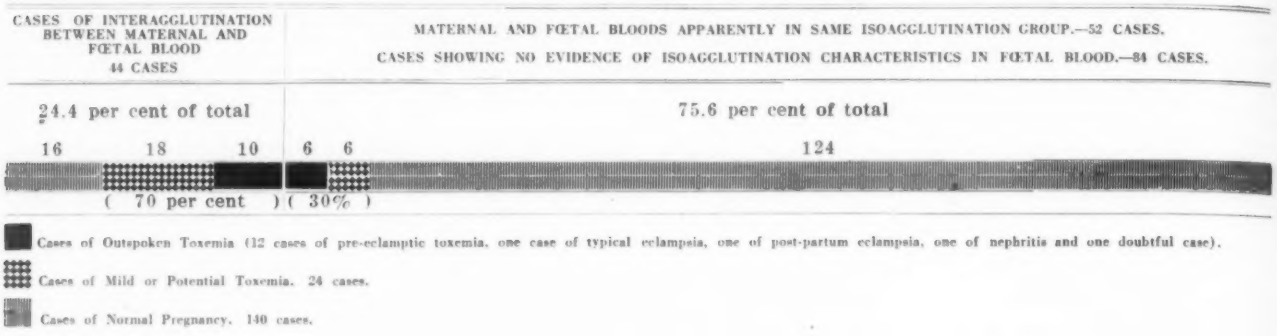
CASES OF MILD OR POTENTIAL TOXEMIA

| Patient's Case No. | CLINICAL SIGNS AND SYMPTOMS | | | | | | Interagglutination | Blood Group | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood Pressure | | Œdema | Albuminuria | Severe Headache | Nausea and Vomiting | | | | |
| | Syst. | Diast. | | | | | | M. | C. | |
| 10936 | 149 | 90 | 0 | Trace | 0 | Early | + | 4 | 2 | |
| 11063 | 142 | 94 | Moderate | 0 | + | 0 | + | 3 | 2 | |
| 11089 | 150 | 90 | 0 | Trace | + | + | + | 4 | 2 | |
| 10984 | 145 | 95 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | Child's blood shows no group |
| 10989 | 145 | 90 | Moderate | 0 | + | 0 | 0 | 4 | 0 | Child's blood shows no group |
| 10827 | 150 | 90 | Slight | Trace | 0 | + | + | 4 | 2 | |
| 10771 | 145 | 90 | 0 | Trace | 0 | 0 | + | 3 | 2 | |
| 11052 | 140 | 95 | Slight | Big trace | + | + | + | 2 | 3 | |
| 10947 | 146 | 100 | Moderate | Big trace | 0 | 0 | + | 4 | 2 | |
| 10955 | 150 | 95 | Slight | Trace | + | + | + | 3 | 2 | |
| 11209 | 142 | 95 | Slight | 0 | 0 | + | 0 | 4 | 9 | |
| 11204 | 148 | 100 | Slight | Trace | 0 | + | 0 | 2 | 2 | |
| 10602 | 146 | 100 | Slight | 0 | + | 0 | + | 4 | 1 | |
| 10607 | 158 | 96 | Marked | + | + | + | + | 4 | 3 | |
| 10839 | 140 | 80 | Slight | 0 | 0 | 0 | + | 3 | 1 | |
| 10843 | 170 | 100 | Moderate | Trace | + | + | + | 2 | 3 | Blurred vision |
| 10866 | 140 | 100 | 0 | 0 | + | + | + | 4 | 2 | |
| 10864 | 150 | 118 | Moderate | 0 | + | + | + | 4 | 3 | Blind spells |
| 10871 | 170 | 120 | Moderate | + | 0 | + | + | 4 | 3 | |
| 10895 | 140 | 100 | 0 | 0 | + | 0 | + | 2 | 1 | |
| 10899 | 140 | 105 | 0 | Trace | + | + | + | 2 | 3 | |
| 19912 | 165 | 105 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | Child's blood shows no group |
| 10635 | 140 | 80 | Slight | Trace | + | + | + | 4 | 2 | |
| 10704 | 140 | 100 | Slight | 0 | 0 | 0 | 0 | 4 | 0 | Child's blood shows no group |

NOTE: 0 = no. + = yes.

CHART I.

Distribution of Normal and Toxemic Cases between the Group showing Interagglutination of Maternal and Fœtal Bloods and that showing no Interagglutination.


CASES OF INTERAGGLUTINATION BETWEEN MATERNAL AND FŒTAL BLOOD
44 CASES
MATERNAL AND FŒTAL BLOODS APPARENTLY IN SAME ISOAGGLUTINATION GROUP.—52 CASES.
CASES SHOWING NO EVIDENCE OF ISOAGGLUTINATION CHARACTERISTICS IN FŒTAL BLOOD.—84 CASES.
24.4 per cent of total
75.6 per cent of total
16
18
10
6
6
124
( 70 per cent )( 30% )
Cases of Outspoken Toxemia (12 cases of pre-eclamptic toxemia, one case of typical eclampsia, one of post-partum eclampsia, one of nephritis and one doubtful case).
Cases of Mild or Potential Toxemia. 24 cases.
Cases of Normal Pregnancy. 140 cases.

*in vitro* tests used, and, in one of the three, cardiac disease with marked œdema throughout pregnancy complicated the picture. The doubtful case of this series, while diagnosticated clinically at first as pre-eclamptic toxemia, showed albuminuria and persistence of arterial hypertension throughout her fourteen days in the hospital following delivery, and should probably be designated as nephritic toxemia. In this instance, as well as in the case of frank nephritic toxemia and also in the one of postpartum eclampsia, the child's blood showed no definite signs of isoagglutination, although in the last instance there appeared to be a very slight tendency to clumping of the child's cells by the mother's serum in two of three tests made. In the case of typical antepartum eclampsia there was strong agglutination and later hemolysis of the child's red blood cells by the mother's serum.

Table IV brings together a group of 24 cases of what may be called mild toxemia, that is, cases showing increases in blood pressure above 140 systolic, (and in all but two elevated diastolic levels as well), associated in most instances with other signs of toxemia. The average systolic pressure in this group was 148.9 mm. of mercury and the diastolic 98.4 mm., whereas the average for the 140 normal cases were 119.6 mm. and 79.6 mm. respectively, while the average of these two groups combined was 123 mm. and 82 mm.

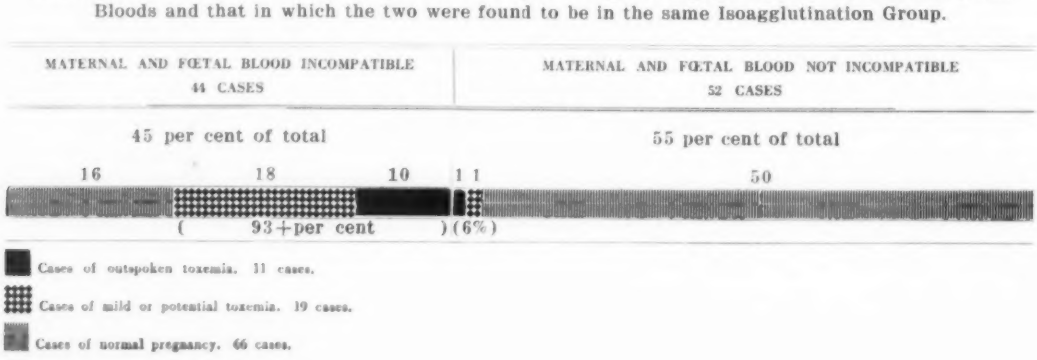
Of the 24 cases tabulated 18, or 75 per cent showed definite incompatibility between the blood of the mothers and of their infants; while 6, or 25 per cent, showed no interagglutination. Of the latter, however, in one case only did the infant belong definitely to the same blood group as the mother, the others showing no recognizable isoagglutination phenomena by the tests made. Just as in the negative cases shown in the preceding table, any or all of the remaining five infants may or may not later be shown to belong to other groups than their mothers. It will be noted from Tables III and IV that none of the infants whose mothers showed signs of toxemia were found to belong to Group IV, which is what would be expected if the theory based upon our data is correct.

Chart I presents a summary of our studies in a graphic way. The spaces at the top of the chart represent the distribution of the 180 cases as regards the phenomena of agglutination, while the lower bar shows the corresponding distribution as regards clinical classification.

The striking feature of these results, as shown by the chart, consists in the great disproportion in the distribution of the cases of toxemia between the two groups as

CHART II.

Distribution of Toxemic Cases between the Group showing Incompatibility of Maternal and Fœtal Bloods and that in which the two were found to be in the same Isoagglutination Group.



regards interagglutination between mother's and infant's blood. Although but 24.4 per cent of the cases studied showed interagglutination, 70 per cent of the cases of toxemia occurred in this smaller group. In other words, while 63.6 per cent of the cases showing interagglutination manifested signs of toxemia, only 8.8 per cent of the cases showing no interagglutination presented similar signs. It is important also to repeat at this point that, in nearly all of the toxemic cases which showed no interagglutination, the child's blood showed no evidence of isoagglutination reactions at all. When such reactions do develop in these individual infants, they may or may not be shown to be in groups other than those of their mothers.

Chart II shows the relative distribution of the toxemic and normal cases between the two groups in which the isoagglutination characteristics of the infant were at least partially established. It shows that the group with incompatibility between the maternal and fœtal blood contains 93.3 per cent of the total number of toxemic cases, as contrasted with 6.7 per cent in the larger group showing compatibility. Although 63.6 per cent of the cases in the incompatible group showed evidence of toxemia, 3.8 per cent only of the compatible cases showed similar signs. In other words, according to the limited data presented in this chart toxemia would appear to be 16.5 times more likely to occur when the maternal and fœtal bloods are incompatible than when they are in the same blood group. Moreover, if the uncomplicated eclamptic and pre-eclamptic toxemia cases alone are considered, the results are even more striking.

It is interesting to compare the average blood pressures of the entire group of cases showing interagglutination between maternal and fœtal blood with those showing no interagglutination. The average for the former, including both clinically normal and toxemic cases, was, systolic 142.8 mm. of mercury and diastolic 92.3 mm., whereas that for the latter was 121.7 mm. and 79.6 mm. respectively. Furthermore, the average pressures for the series in which the child's blood group was partially established and was found to be the same as the mother's, were 119.2 and 78.5 mm. Blood pressure values are selected for this comparison because they are sharply marked and can be expressed in figures. However, the other signs appear to follow the same general variations of incidence as the blood pressure.

## Discussion

The results submitted here, so far as they go, are suggestive of the existence of some relationship between incompatibility of the blood group of the pregnant woman with that of her fœtus, and the development of eclampsia or pre-eclamptic toxemia. When the clinical features of blood transfusion reactions are compared with those of obstetrical toxemias, a certain similarity between them must be admitted. Moreover, if the results of animal experimentation, such as those cited, are considered, the underlying pathology in the two conditions likewise appears to present many features in common.

Before it is possible, however, to consider that any form of toxemia or reaction can result from interagglutination of maternal and fœtal blood the following requirements must be fulfilled: (1) that the isoagglutination characteristics of the fœtus be developed at the time the toxemia appears; (2) that the fœtal blood grouping differ from that of the mother; (3) that a break in the placental barrier between the two circulations occur at some point, and (4) that sufficient fœtal blood enter the maternal circulation to give rise to the clinical and pathological changes observed.

While there are practically no data available concerning the earliest appearance of isoagglutination or isohemolytic characteristics in fœtal life, reference to Table I shows that such are present in a surprising number of new-born infants, some of which are premature. Consequently, it may reasonably be assumed that in some cases the blood is capable of such reactions early in the last quarter of pregnancy, or perhaps earlier, that is, at the time when the toxemia is also prone to occur.

That the child's blood grouping may differ from that of its mother is conclusively shown by this and other reports, and is what would be expected from our present knowledge of the stability and transmissibility of the blood groups as hereditary characters.[35,36,37]

While no constant relationship has been observed between the occurrence of toxemia and infarction or premature separation of the placenta in these cases, the recent work of Young[38,39] on the etiology of eclampsia is very instructive in this connection. After careful observation of a considerable number of cases of eclampsia and pre-eclamptic toxemia with close study of the placenta, he concludes that the toxemia is due to premature separation of the placenta or to placental infarction with subsequent absorption of toxic products from such areas. If his conclusions concerning the mode of production of toxemia are correct, they provide a possible explanation as to the fœtal source of the agglutinated red blood corpuscles referred to by Flexner as composing the hyaline thrombi in the liver.

The question next arises regarding the possibility of a sufficient quantity of incompatible blood passing from the fœtal to the maternal circulation to give rise to a reaction in the mother, when it is remembered that even late in pregnancy the fœtal blood volume does not exceed 300 c.c to 500 c.c.[40] This seems entirely possible under certain circumstances, since, as pointed out by Ottenberg,[22] a few large clumps of foreign cells tend to cause a more serious reaction than a much larger number of cells in small clumps. Such a heavy clumping is what might be expected with the gradual entrance into the maternal circulation of fœtal red blood cells strongly agglutinable by

mother's serum. Schultz [41] found that 50 c.c. of a donor's blood, for which the recipient's serum was hemolytic and agglutinative, was sufficient to cause fever, vomiting, diarrhea, œdema of the hands and face, and finally collapse. Furthermore, it is a common observation that reaction to blood transfusion, where it does occur, manifests itself very early in the procedure, and occasionally after as little as 20 c.c. to 25 c.c. have been given. It is, therefore, quite conceivable that with sufficient separation of the placenta a living fœtus might pass from one tenth to one sixth of its total blood volume into the maternal circulation within a comparatively short time. In this event the severity of the reaction on the part of the mother might conceivably be dependent upon the amount of fœtal blood thus transfused, as well as upon the strength of the agglutination.

If one entertains the possibility of such a relationship as has here been suggested, it will be necessary to provide some other explanation for the smaller group of toxemia cases in which there is no interagglutination between the maternal and fœtal blood. After allowing for experimental errors, the following possibilities might be considered.

In the first place, it is probable that some of the cases diagnosticated as the pre-eclamptic or eclamptic type of toxemia are really not such. These naturally need not be discussed in this connection, because they might be expected to fall into either group according to the law of chance.

It is very important to note that in all of the negative cases but two, the infant's blood showed no sign of isoagglutination phenomena *in vitro*, and in them the blood grouping of mother and child may or may not later be shown to be identical. Moreover, it is a well recognized fact that minor toxic reactions frequently follow direct transfusion of blood, even when careful *in vitro* tests appear to show that the donor and recipient belong to the same general isoagglutination groups, thus indicating that our *in vitro* methods are not sufficiently refined to detect such differences. Finally, there remains the possibility that the difference in blood grouping of mother and fœtus is only an index to a fundamental dissimilarity between the body proteins of the two, making the development of an anaphylactic reaction possible under certain circumstances in accordance with the suggestions of Rosenow and Anderson,[42] Gräfenburg,[43] Vertes,[44] and others, regarding the anaphylactic nature of eclampsia. It would be difficult, perhaps, on any other basis than the latter to account for the very rare cases, described in the literature, of eclampsia occurring during the early months of pregnancy and in women harboring an hydatidiform mole instead of a fœtus.

Regarding the cases showing interagglutination but no signs of toxemia, one can only suggest that in such instances the placental barrier between the maternal and fœtal circulations has remained intact, or that some yet unknown defensive mechanism exists, which prevents the injurious reaction.

Should the suggestions contained in this report be substantiated, postpartum eclampsia would have to be regarded as a "delayed reaction," the blood having passed from the fœtal to the maternal circulation when placental separation occurred at birth or shortly before, but without manifesting its most striking effects until a few hours later, time being required for development of thrombi and lesions in the liver, the kidneys, the brain and elsewhere. Furthermore, it might be suggested that the existence of double ovum twin pregnancy predisposes to the development of toxemia largely for the reason that it offers a greater chance for incompatibility between the fœtal and maternal blood than in a single pregnancy.

The numerous theories regarding the etiology of eclampsia cannot be discussed here. For their comprehensive, critical discussion and for a good general bibliography on the subject, the excellent chapters on the toxemias of pregnancy in Williams' "Obstetrics," [45] and Kosmak's recent monograph,[46] should be consulted. It may be observed in passing, however, that profound disturbances of metabolism, as well as symptoms directing attention to the kidneys or to the brain, might be merely incidental features of the reaction and secondary to the underlying changes initiated by the transfusion of incompatible fœtal blood.

Many of the suggestions noted in this brief discussion appear to be susceptible of substantiation or disproof, and further studies are planned for their solution. Confirmary evidence might, for instance, be adduced if fresh blood smears from eclamptic patients could be studied at various stages and be shown to contain agglutinated red blood cells, similar to those referred to by Bernheim [20] in a case of transfusion with incompatible blood.

## Summary

1. The isoagglutination reactions of 180 women, 54 white and 126 black, and those of their new-born infants have been studied.

2. The infants' blood group was apparently completely established in 20 of the cases, or 11 per cent, and partially established in over half of the cases.

3. The red blood corpuscles of the new-born infants possessed receptors twice as frequently as the corresponding sera contained agglutinins.

4. The mother's serum agglutinated her own infant's red blood cells in 23.3 per cent of the cases studied, whereas the infant's serum agglutinated the mother's red blood cells in but 2.7 per cent.

5. In 28.8 per cent of the cases infant and mother apparently belonged to the same isoagglutination group, while in 46.6 per cent there was no evidence of isoaggl-

tinins in the serum and no receptors on the red blood cells of the infant.

6. The distribution of the colored patients in the four isoagglutination groups was odd, in that there were none in Group I and more in Group III than in Group II.

7. It was found that over 70 per cent of the cases of toxemia (all forms included) occurred in the small group in which there was interagglutination between maternal and fœtal blood.

8. Toxemia occurred 16.5 times more frequently when the maternal and fœtal bloods were demonstrated to be incompatible than when they were in the same isoagglutination groups.

### CONCLUSIONS

1. The isoagglutination blood group is completely established at the time of birth in a small percentage of cases and partially established in a relatively large percentage.

2. The intact placenta is impermeable to isoagglutinins, and the blood group of the infant may differ from that of its mother.

3. Before the mother's blood or that of any other individual is employed for transfusion of an infant, it should in every case be matched with that of the infant as a preliminary precaution, to avoid with safety dangerous reactions.

4. Although the data submitted in this paper are not sufficient to warrant the drawing of final conclusions, they are very suggestive of the existence of some relationship between the incompatibility of maternal and fœtal blood on the one hand and the development of eclampsia or preeclamptic toxemia on the other.

5. Such a theory, however, should be regarded as merely tentative, and cannot be accepted until a satisfactory mechanism has been demonstrated by which the fœtal blood can gain access to the maternal circulation.

NOTE: It is a pleasure to express my gratitude to Dr. Williams and to Dr. Guthrie for their interest and assistance in this work.

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# PERIODIC VARIATIONS IN SPONTANEOUS CONTRACTIONS OF UTERINE MUSCLE, IN RELATION TO THE OESTROUS CYCLE AND EARLY PREGNANCY

By JOHN D. KEYE

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## INTRODUCTION

The reaction of isolated preparations of uterine smooth muscle to various drugs has long been known to be variable, so much so that it is considered necessary in current pharmacological studies to standardize these preparations by using the virgin uterus alone. The cause of this variation remains obscure, but during the course of some work on smooth muscle elsewhere in the reproductive tract by two fellow-workers of the writer (Guttmacher and Guttmacher, 1921) the suggestion was made that the variability of the mature uterus might be a function of the œstrous cycle. The writer undertook to test this suggestion, but the first experiments disclosed a still more interesting fact, namely, that there is a regular variation in the rate, amplitude and type of the spontaneous contractions of the isolated uterine muscle, both during the œstrous cycle and in early pregnancy.

Beyond a note published by Blair (1922), while our work was in progress, there appears to be no previous knowledge concerning this phenomenon. Blair's work, which was done on the albino rat, will be discussed later in connection with our own results.

The animal chosen for our work was the domestic pig, for the reason that the sequence of the œstrous cycle and the complete histological picture of both ovary and uterus have been so thoroughly worked out that specimens obtained from the abattoir can be dated with accuracy as to their place in the cycle. Further, the demonstration of a physiological change coincident with the histological variations could not fail to be useful by helping to complete our knowledge of a chain of events which is already beginning to be well known in this species.

The arrangement of apparatus was that commonly used in the study of muscular contractions. A transverse strip of uterine wall 6 mm. wide by 30 mm. long, suspended in oxygenated Locke's solution at 37.5° C., was attached to the short arm of a recording lever. The kymograph drum revolved once in sixty minutes. Within an hour from the time the animal was killed the experiment was under way. The first half hour of the record usually showed nothing more than a rise probably indicating a recovery of tonus, but in some instances the periodic contractions started immediately. Because of this fact the first thirty minutes of the records were disregarded with the idea that by the end of that time all specimens had attained their optimal condition. The final reading was taken from the ensuing period. The tubes and uterus of each specimen were carefully washed out with saline to recover any eggs or early embryos. Sections of ovaries and uterus were made for microscopic study and were assigned to their correct date in the cycle according to the method worked out by Corner (1921b), which is briefly as follows:—During the 21-day cycle in swine, the first three days following ovulation are marked by the presence of freshly ruptured Graafian follicles (early corpora lutea) in the ovaries, and the ova may be found in the Fallopian tubes. From the fourth to the seventh day the ova are in the uterus and may be recovered therefrom by washing. By the seventh day the corpora lutea are solid, having reached a diameter of 9 mm., and the ova disappear from the uterus by degeneration about the seventh or eighth day. About the end of the second week the corpora suddenly begin to degenerate, as shown by their diminution in size, increase in firmness, and change from flesh to yellowish color. Microscopically, the granulosa-lutein cells are degenerating. During the latter part of the third week the new Graafian follicles enlarge from the resting stage to a diameter of 8 to 10 millimeters; following their rupture the cycle begins anew. As shown in the above-mentioned monograph, a striking series of histological changes is in progress in the uterus during the cycle. By aid of these data all specimens used were dated as to their place in the cycle. In the case of early pregnancies the data furnished by Assheton (1899) and by Keibel (1897) were used.

Observations were made upon a series of preparations as follows: animals taken during œstrus, 5 (of these 2 had unruptured follicles, 3 had ova in tubes); fourth to seventh day (ova in uterus) 6; eighth to eleventh day, 5; twelfth to fifteenth day, 4; sixteenth to twentieth day, 2. Total 22.

Pregnancies: Sixth day, 1; ninth day, 1; thirteenth day, 1; fourteenth-fifteenth day, 2; twentieth day, 1; 10 mm. embryos, 1; 15 mm. embryos, 1; 21 mm. embryos, 1. Total 9.

Immature animals, 4.

## OBSERVATIONS

Examination of records taken in the non-pregnant cycle discloses that the graph of uterine contraction is made up of two types of waves, namely, major waves of relatively long duration (1.5 to 2.5 minutes) and minor waves of

higher frequency, superimposed upon the former. These two types of waves vary in inverse relation to each other as the cycle advances from œstrus through the interval to another œstrus, in the following manner: at the time of heat when the follicles have just ruptured and the discharged eggs have first entered the tubes (Fig. 2), there is an absence of the minor contractions while the major contractions are few in number but strong. A day later when the ova are in progress through the tubes (Fig. 3), the major contractions increase in rate while the minor are just beginning to appear. By the time the ova are in the uterus (Fig. 4), a mean is reached, there being regular contractions of both types in such a manner as to make the tracing resemble the well-known cardio-respiratory waves of an arterial tracing. About the seventh day the ova are degenerating in the uterus; this is accompanied by a physiological change wherein the major contraction waves almost disappear and only the minor are present, these latter showing an increased frequency (Fig. 5). In the next later period (Fig. 6) 8 to 10 days *post œstrum*, the maximum rate of the minor contractions is reached, the major contractions being in abeyance. The most quiescent stage is reached by the 13th day (Fig. 7), both types of waves being present, but with a marked decrease in activity. About the 15th day the major contraction waves again become evident, so that the tracing once more resembles the cardio-respiratory waves of a pulse-tracing (Fig. 8). This stage is followed by enlargement of the Graafian follicles about the twentieth day (Fig. 1), at which time the major contractions reach their maximum, the minor rapidly disappearing.

Pregnancy definitely alters the type of cyclic contractions. It may be recalled that when fertilization occurs in the sow, the segmenting ova pass into the uterus at about the fourth day; from the fourth to about the twelfth day they are present in the uterine cavity as freely floating blastocysts. During this time (as shown by Corner, 1921a) the embryos are being shifted to their final resting places in the uterine cavity, some of them at times even being transported by internal migration into the opposite uterine cornu, thus making a journey which may amount to 100 centimetres. By the twelfth day the great elongation of the chorions, which is characteristic of the pig, is well advanced and by the fifteenth day the chorions are 30 centimetres in length. At some time between the tenth and fifteenth day, not capable of exact definition, the embryos have become evenly spaced in the cornua, and the gradual enlargement of the chorions has resulted in approximating the chorionic surfaces to the uterine epithelium, so that they may be said to have become implanted.

If we may trust our few tracings of the uterine contractions taken from animals containing free blastocysts (Fig. 9), it appears that the change in contraction-type occurs as early as the 6th day of pregnancy. It consists of a quieting of both types of contraction waves, more particularly the minor type, which as we have described is in evidence in non-pregnant animals at the same time after œstrus. This deadening of the contractions lasts until the chorions have become elongated (Figs. 10 and 11), when both types of contraction gradually appear once more, though not reaching a state of full activity. The minor contractions apparently cease about the 21st day of pregnancy, and thereafter (Figs. 12 and 13), as far as our series of observations goes, (through the first third of gestation) the major contractions alone continue, and even these are few and irregular.

Immature animals (Fig. 14) all give one type of contraction curve characterized by the fact that the larger waves, which alone are present, show a rate of contraction much faster than at any time in the mature animal's cycle, but not quite so extensive as the most active contractions of the mature œstrous uterus. This type of contraction is so constant that it no doubt is comparable to the constancy of uterine activity in young virgin guinea-pigs, which has led pharmacologists to use the latter tissue for the purpose of standardizing certain drugs acting upon smooth muscle.

## DISCUSSION

1. Blair, working on the albino rat, found a definite variation in the uterine contraction rate, stating that the rhythm is found to be slowest at the time of œstrus, increasing gradually to a maximum late in the resting stage and decreasing rather suddenly at the pro-œstrus. The rhythm of the resting uterus is often broken up by the appearance of superimposed contractions before relaxation is complete. These were ignored in estimating the rate. Barely perceptible minimal contractions were often found interpolated between the ordinary maximal contractions of the uterus at and near œstrus. The difficulty of attempting to make a direct comparison between the uterine cycle of the rat and the domestic pig is at once apparent. The former has a cycle of but four to six days' duration, in which the corpora lutea from one ovulation overlap and continue into the next period, and pregnancy has a complicated effect upon this order of events (Long and Evans, 1922). In the sow one is dealing with an animal which ovulates every 21 days and in which the appearance and disappearance of corpora lutea and Graafian follicles take place in well separated stages which do not overlap each other or those of the succeeding ovulation; thus ample time is given for the full development of the physiological events of each ovulation cycle.

Blair speaks of maximal and minimal contractions, but whether they correspond to our similar types as found in the sow, one would hesitate to say. However, the minimal contractions of the rat's uterus, since they are present, according to Blair, during the resting stage of the cycle, are thus presumably comparable to the minor contractions which are in evidence in the sow from the 6th to the

15th day after ovulation. Beyond this suggestion it seems difficult to mark off any further parallelism between the two species.

2. The periodic occurrence of these two types of contraction curves in the normal uterine cycle of the sow calls forth a very interesting comparison with the anatomical changes taking place in the ovaries of the same animal as described by Corner (1921b), in his work on the relation between œstrus, ovulation and the development of the corpora lutea in the sow. Referring once more to that study, one sees that the growth and bursting of the Graafian follicles occupy about 4 days, preceding and during œstrus. The corpora lutea reach their maximal growth at about the 7th day after ovulation and continue without degeneration until about the 14th or 15th day, when they rapidly retrogress, and in a short time the cycle begins anew. From the physiological study we find that coincidentally with the growth and bursting of follicles and for a short time afterwards the major contractions predominate; they alone being present when the ova are in the tubes, or just after the follicles have discharged their contents. The minor contractions apparently begin with the growth of the corpora lutea, continuing to increase in number until about the 13th day, when their maximal rate is reached, and they alone are present. From the 14th day on, they decrease in rate and the major waves begin to be in evidence. By the 20th day the greater contractions are almost at their height of activity, while the lesser are rapidly diminishing, and the cycle begins anew. Figure 15a is a diagram showing the relative increase and decrease in rate, amplitude, and general activity of the two types of uterine waves described above, arranged for direct comparison with Corner's diagram (Figure 15b), which shows the relations between œstrus, ovulation and the development of the corpora lutea in the sow. As will be noted, the presence of the mature corpora lutea is coincident with the maximal development of the minor contraction waves, while the period of retrogression of the corpora lutea, maturation of new follicles, and earlier stages of the new corpora lutea, is parallel to the maximal development of the major contraction waves. Twice in the cycle, namely, about the 6th and 15th days, as shown on the appended diagram at the points where the two curves cross, both types of contraction are present to a medium degree, so that the tracing presents a compound or "cardio-respiratory" curve. As indicated by the abrupt slope of the diagrammatic lines, neither type of wave is totally absent for any significant length of time.

The observations recorded in this paper are of especial interest as indicating a possible solution of the much discussed problem as to how the ova and early embryos are transported through the uterine canal and arranged in regular order for implantation. This function has generally been ascribed to the cilia lining the reproductive tract, but the probable incorrectness of this view has been pointed out by Sobotta (1916) for the Fallopian tubes, and emphasized as regards the uterus by Corner and Snyder (1922), who showed that in this species the epithelium of the uterus, with the exception of that in the endometrial glands, is not ciliated; and also that there is not, as is often claimed, a cyclic variation in ciliary activity. Furthermore, it is known that at times in the pig free blastocysts move down one horn of the uterus and up the other; in such a case the movement must in part be against the ciliary current (Corner 1921a). The only other forces capable of transporting and spacing the ova and early embryos would be chemotaxis (for which there is no evidence), or a peristaltic action of the uterine musculature. For this last hypothesis it seems that the results reported in this paper offer strong presumptive evidence. By analogy with the contractions of isolated preparations of intestinal muscle *in vitro*, the contraction of a strip of uterine muscle, as described in this paper, may be considered as representing a peristaltic action of the uterus; moreover, we have seen that the uterine contractions are modified according to a schedule which closely parallels the events of the ovulation cycle.

## Summary

1. In the pig there is a definite physiological change in the uterine musculature accompanying and coincident with the histological changes which take place in the internal genitalia during the œstrous cycle.

2. There are two types of spontaneous contractions present in the isolated uterine musculature, *viz:*—relatively large contractions of long duration (1.5-2.5 minutes), and smaller contractions of briefer duration which are superimposed upon the former.

3. During the time the Graafian follicles are maturing and for a short time after they burst, contractions of the major type predominate or are present alone.

4. When the corpora lutea reach maturity and up to a time shortly before they retrogress, the minor contractions predominate or are present alone.

5. These contractions (which are probably of a peristaltic nature) may by their variations be the active agency in transportation of ova and early embryos to their final position for implantation in the uterus.

6. The intervention of pregnancy alters the physiological picture, by causing marked diminution in both types of contraction waves, particularly the minor type.

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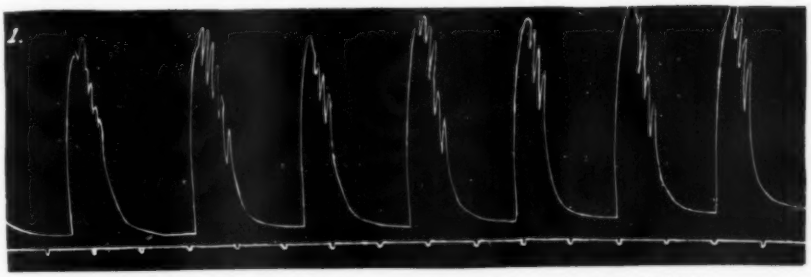


Fig. 1.—Immediately before ovulation; mature Graafian follicles.

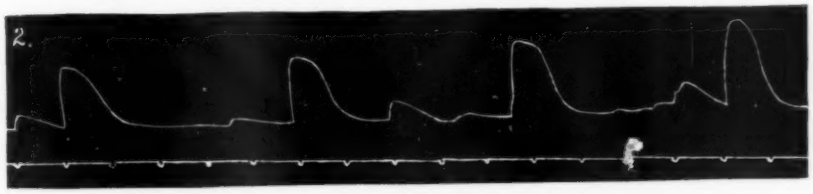


Fig. 2.—Ovary contains recently ruptured follicles.

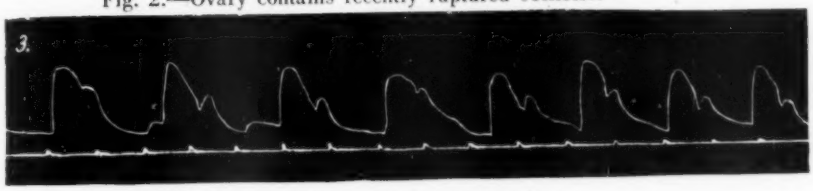


Fig. 3.—2d day after ovulation; ova in tubes.

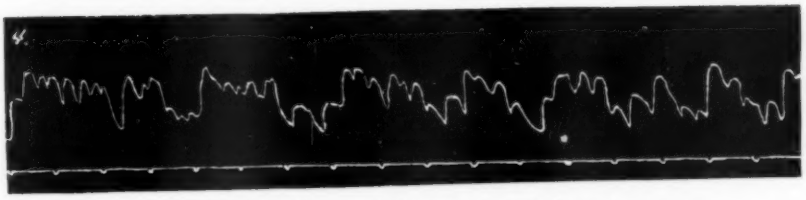


Fig. 4.—Ca. 4th day after ovulation; unsegmented ova in uterus.

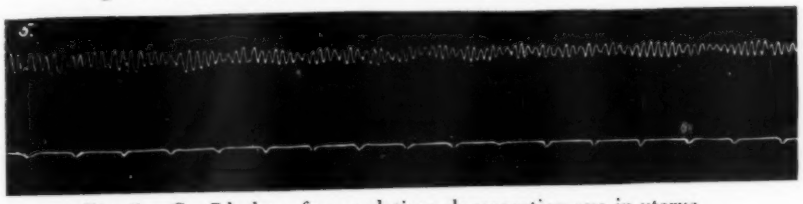


Fig. 5.—Ca. 7th day after ovulation; degenerating ova in uterus.

Fig. 6.—8th to 10th day after ovulation.

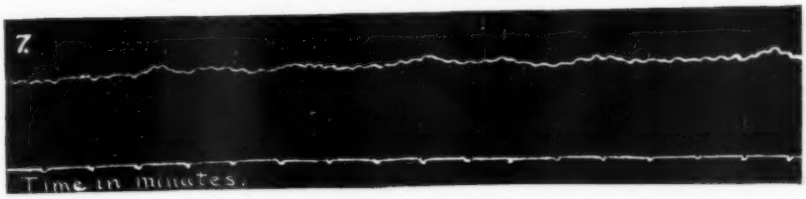


Fig. 7.—12th to 13th day after ovulation.

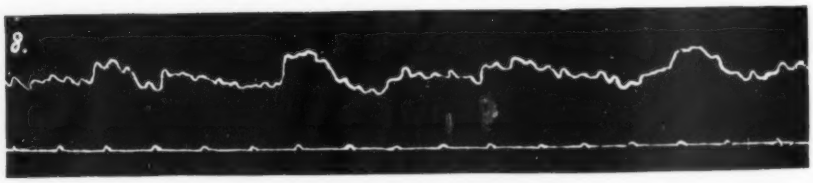


Fig. 8.—15th to 16th day after ovulation.

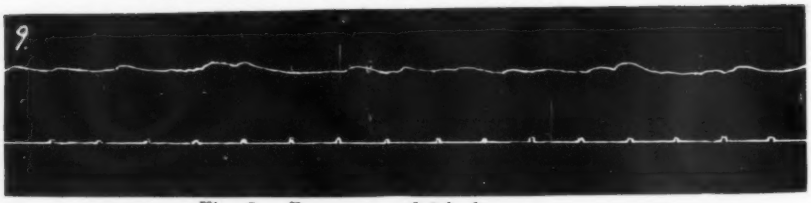


Fig. 9.—Pregnancy of 9th day.

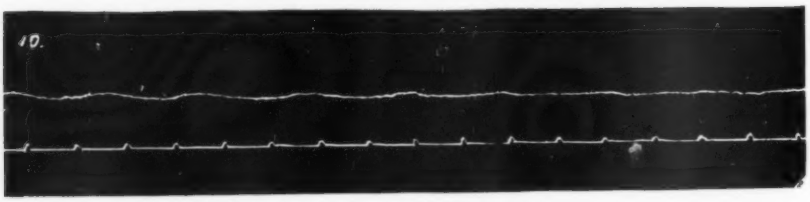


Fig. 10.—Pregnancy of 13th day.

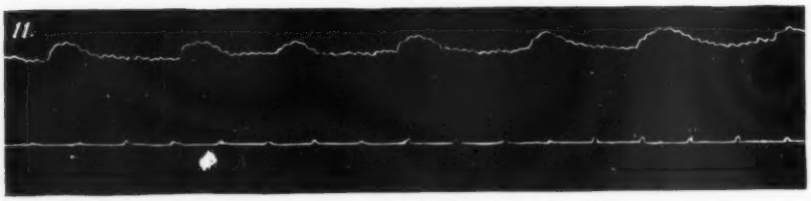


Fig. 11.—Pregnancy of 18th day.

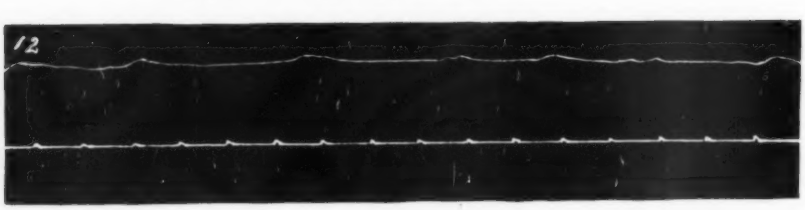


Fig. 12.—10 mm. embryos.

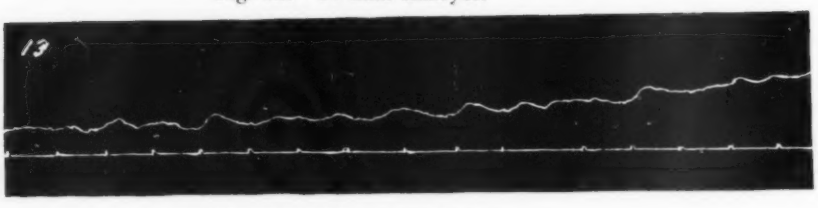


Fig. 13.—21 mm. embryos.

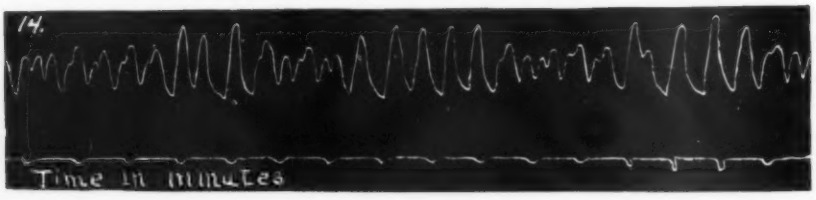


Fig. 14.—Immature animal.

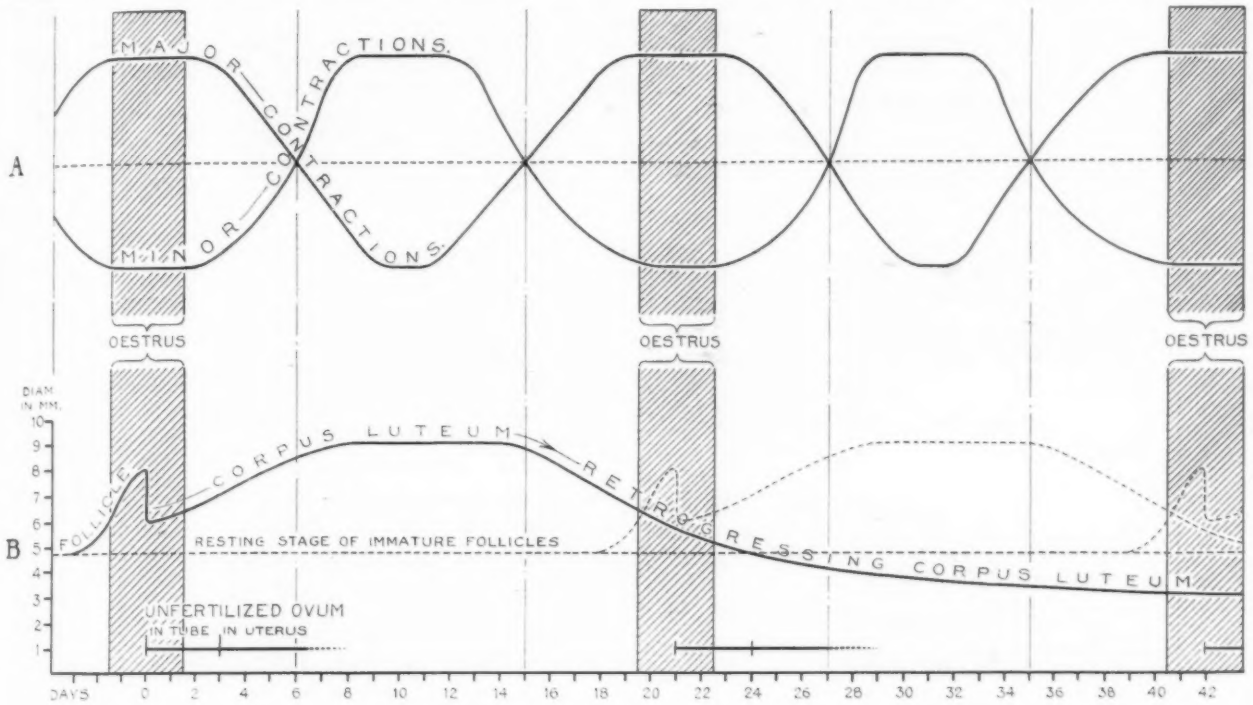


Fig. 15.—Diagram showing in graphic form the relative increase and decrease in rate and amplitude of the two types of contraction waves throughout the oestrous cycle of the sow, in comparison with a diagram showing the relations between oestrus, ovulation, and the development of the corpus luteum in the sow.

*Idem:* 1921b. Cyclic changes in the ovaries and uterus of the sow, and their relation to the mechanism of implantation. Publ. Carnegie Inst. Washington, No. 276, (Contrib. to Embryol. vol. 13), 117-146.

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# THE PREPARATION OF NUCLEOTIDES FROM YEAST NUCLEIC ACID

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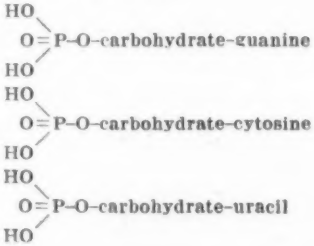
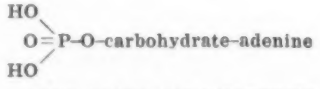
Received for publication January 25, 1923.

It is widely believed that yeast nucleic acid is a tetranucleotide composed of the groups of four mono-nucleotides joined to one another in some unknown manner called the nucleotide linkages.



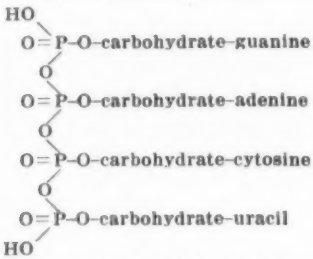




THE FOUR NUCLEOTIDES

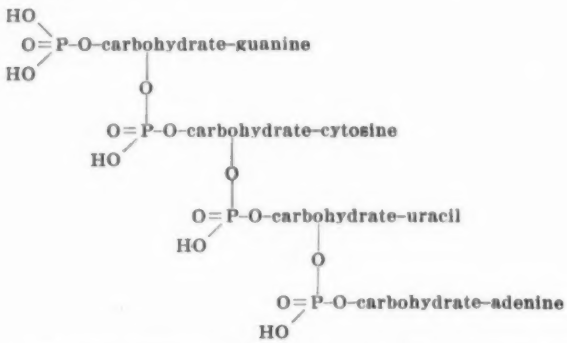
(The names are intended to represent groups)

Modern arguments on the constitution of yeast nucleic acid have been mostly confined to the location of the three nucleotide linkages which bind the four nucleotide groups to one another. The original assumption [1] (based on no experimental evidence whatever) was expressed in the following formula:

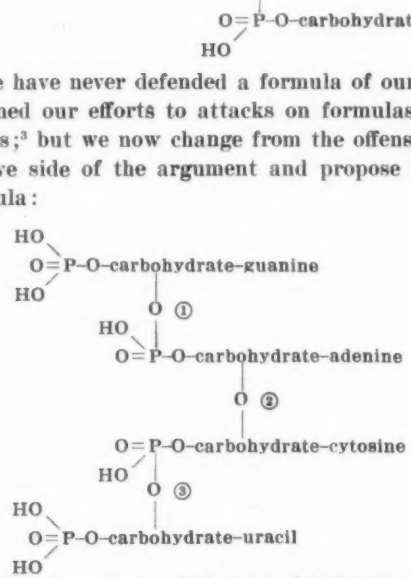
[1] Levene, P. A., Biochem. Zeit., 1909, XVII, 120.




in which the three nucleotide linkages are exerted through the phosphoric acid groups. But in the face of the first opposition this formula was abandoned and the following one proposed [2] (also on little or no experimental evidence):




We have never defended a formula of our own, having confined our efforts to attacks on formulas proposed by others;[3] but we now change from the offensive to the defensive side of the argument and propose the following formula:




Neither the order in which the nucleotide groups are arranged nor even the identity of the groups is intended to be significant.

Acceptance of this formula would concede the following:

(1) A rupture of nucleic acid at one of its nucleotide linkages (No. 2) would not give rise to any increase in titratable acidity (neither toward methyl orange nor toward phenolphthalein). It would produce two di-nucleotides both of which *may* be precipitable by the addition of hydrochloric acid, and not by sulphuric acid.

(2) A rupture of nucleic acid at either or both of two of its nucleotide linkages (Nos. 1 and 3) would give rise to an increase in acidity to phenolphthalein but not to methyl orange. It would also produce a di-nucleotide which *may* be precipitable by the addition of hydrochloric acid and not by sulphuric acid.

(3) No rupture of any nucleotide linkage should give rise to acidity toward methyl orange.

The experimental evidence which bears on the subject and for which there is no other apparent explanation is as follows.

A fresh aqueous extract of pancreas contains a multitude of ferments; but when the extract is boiled, all are destroyed save one, namely, the one that decomposes nucleic acid. So that if one digests nucleic acid with a boiled aqueous extract of pancreas, the nucleic acid disappears and under the following conditions:

(1) No phosphoric acid is split off.
(2) No purine base is split off.
(Two propositions that are proved by the application of two obvious chemical tests.)
Hence *only nucleotide linkages are ruptured.*
(3) No titratable acidity appears (even to phenolphthalein).
(4) The product gives no precipitate with sulphuric acid. It cannot therefore contain any undecomposed nucleic acid.
(5) The product does give a bulky precipitate with hydrochloric acid. It cannot therefore be composed of simple mono-nucleotides.
(6) By the application of proper analytical procedures, all four of the mono-nucleotides can be isolated from the product. But they are formed in the chemical manipulation of the material, as will readily be granted.

It is therefore fair to conclude that the nucleotide linkage No. 2 of yeast nucleic acid is ruptured by the action of boiled pancreas.

Again, we have recently discovered an adaptation of a chemical process by which yeast nucleic acid can be decomposed rapidly and quantitatively into its nucleotides and under the following conditions:

(1) No phosphoric acid is split off.
(2) No purine base is set free.
(3) Titratable acid (to phenolphthalein but not to methyl orange) *is* produced from the beginning.
(Rupture of nucleotide linkages Nos. 1 and 3.)
(4) If the decomposition is interrupted after half the time required for completion, the product will be found precipitable by hydrochloric acid but not by sulphuric. Here again is suggested an initial rupture of linkages Nos. 1 and 3 forming a di-nucleotide.
(5) After the lapse of several hours, the product consists evidently of simple nucleotides,

[2] Levene, P. A., J. Biol. Chem., 1919, XL, 415.

[3] Jones, Walter, Amer. J. Physiol., 1920, LII, 193 and 203; J. Biol. Chem., 1922, L, 323.

for it can not any longer be precipitated by hydrochlorid acid. Thus it appears that the chemical agent first splits nucleotide linkages Nos. 1 and 3 and afterwards linkage No. 2, giving rise finally to the simple mononucleotides.

This chemical decomposition of yeast nucleic acid places in our hands a method by which the nucleotides of nucleic acid can be quickly gotten into our possession in pure form and in any desired quantity. We naturally propose to establish the identity of these nucleotides and to find whether the uracil group is actually present in the nucleic acid molecule or whether all uracil derivatives hitherto obtained from nucleic acid (including uracil, uracil nucleoside and uracil nucleotide) are laboratory products referable to the deaminization of their corresponding cytosine precursors.

But the easy access of unlimited quantities of these substances affords an opportunity to study the physiological decomposition of nucleotides; a line of investigation from which we have hitherto been excluded for lack of the necessary material.

The chemical method referred to has been fully examined and would be reported in detail at this time but that some analytical data are still lacking. It will be published in the very near future.

# THE EFFECT OF ANTISEPTICS ON THE BACTERIAL FLORA OF THE UPPER AIR PASSAGES

By ARTHUR L. BLOOMFIELD, M. D.

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## INTRODUCTION

The disappointing results of immunotherapy in many acute and chronic infections has led to extensive chemotherapeutic endeavors. The upper air passages in particular, owing to their ready accessibility by the methods of exploration of the rhinologist, have furnished a field for experiment, and in practice the swabbing of infected or inflamed areas in the nose or throat with various antiseptics may be said to have become a routine. Numerous attempts to clear up respiratory "carriers" by similar procedures have also been made. Since the results of treatments of this sort have been interpreted largely on the basis of general clinical impressions, it seems desirable to determine definitely the actual effects of various antiseptics on the bacterial flora of the upper air passages. In the present work an attempt has been made to obtain such information. The experiments will first be presented and will then be followed by such clinical conclusions as seem justifiable.

## LITERATURE

The literature on the effects of antiseptics on bacteria in the upper air passages concerns itself mainly with attempts to eliminate particular organisms from carriers. In the case of diphtheria bacillus carriers such antiseptics as Dobell's solution, tincture of iodine, hydrogen peroxide, potassium permanganate, silver nitrate, argyrol, protargol mercurochrome and gentian violet have been tried. In meningococcus carriers, in addition to the above, chloramine T (by inhalation or by spray), acriflavine, formalin and guaiacol have been extensively used. The reported results are difficult to interpret. It must be remembered that unless the individual has been proven to be a chronic carrier before treatment is begun, the possibility always remains that the cessation of the carrier state may be spontaneous and not due to the antiseptic. Fildes and Baker[1] report careful studies on 632 meningococcus carriers in which attempts were made to check the carrier state by the use of various antiseptics. They state that "looking at the results as a whole we conclude that one-third of the men recover spontaneously and that none of the methods tested have any conspicuous merit." Glover[2] also reports interesting experiments carried out in the British army on the effect of inhalations of 2 per cent chloramine and 0.5 per cent zinc sulphate on meningococcus carriers. The impression was gained that repeated inhalations led to the clearing up of temporary and in some cases of chronic carriers, although the observations were made under conditions where a marked spontaneous fluctuation in the carrier state occurred. During the last ten years we have made numerous attempts to rid chronic diphtheria bacillus carriers of the organisms by applications of strong silver preparations and other antiseptics, *with failure in every case.* In most of these patients the carrier state was finally terminated by tonsillectomy. On the whole, then, the value of antiseptics as exterminators of bacteria in the upper air passages seems in doubt and more exact bacteriological information is desirable.

## EXPERIMENTS

Certain antiseptic drugs representative of those used in rhinological practice were studied. They included potassium permanganate, silver preparations, mercurochrome—220, and another powerful mercurial germicide, designated as meroxyl, which was furnished through the courtesy of Dr. Hugh H. Young. It does not seem profitable to discuss the theoretical mode of action of the various drugs or to review the test-tube experiments on their germicidal power, inasmuch as the actual experi-

mental conditions seemed fundamentally distinct from those which could be reproduced outside the body. We were interested purely in what could be accomplished *in situ* in the way of modifying the mouth and throat flora.

The general plan of procedure was as follows. A swab rubbed over the area to be studied was plated on a series of blood agar and oleate hemoglobin agar plates in such a way as to yield a discrete spread of colonies which allowed easy fishing and counting. The drug was then applied, and cultures were subsequently made and studied in the same way as the control. Notes of both qualitative and quantitative changes following the applications were made.

The first group of experiments dealt with the clinically normal mucous membranes. The tongue was selected as the region most accessible to study. The mouth was first thoroughly rinsed with water to remove any gross foreign particles. A piece of rubber dam with a window about 2 cm. square was then placed on the tongue and the exposed area, which was thus prevented from becoming contaminated by the mouth secretions, was studied.

EXPERIMENT 1.—Dec. 14, 1921. Normal subject.

*Procedure:* 10 per cent mercurochrome—220 was applied to the tongue for two minutes. Cultures were made before application, immediately afterwards, and twenty-four hours later. The results were as follows:

| *Control* | *Culture after application* | *Culture 24 hrs. later* |
|---|---|---|
| (1) * ∞ M. Tetragenus | (1) ∞ M. tetragenus | (1) ∞ M. tetragenus |
| (2) Many non-hemolytic streptococci | (2) ∞ Non-hemolytic streptococci | (2) ∞ Non-hemolytic streptococci |
| (3) Hemolytic influenza bacilli | | (3) A few hemolytic influenza bacilli streptococci |
| | | (4) A few Gram-negative cocci |

* ∞ = innumerable.

*Comment:* The total number of colonies obtained immediately after application of 220 was about three times that recovered from the control. All the colonies remained small, however, possibly owing to an inhibitory effect of mercurochrome carried over on the swab. There was no evidence of any bactericidal effect.

EXP. 2.—Jan. 13, 1922. Normal subject.

*Procedure:* 10 per cent mercurochrome—220 was continuously applied to the tongue for five minutes. At the end of this time the excess dye was removed by irrigating with a liter of water. The results of the cultures were as follows:

| *Control* | *Culture immediately after application* | *Culture 24 hrs. later* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) A few cols. M. tetragenus | (1) ∞ M. tetragenus |
| (2) ∞ Gram-negative cocci | (2) A few cols. Gram-neg. cocci | (2) Many Gram-neg. cocci |
| (3) Several hundred hemolytic influenza bacilli | (3) ∞ Green streptococci | (3) Many hemolytic influenza bacilli |
| (4) Many green streptococci | (4) A very few grey streptococci | (4) ∞ Green streptococci |
| (5) Many grey streptococci | | (5) Many grey streptococci |

*Comments:* The plates made immediately after application showed practically pure cultures of green streptococci. The total number of colonies was vastly increased but the individual colonies were small and stunted. The remainder of the flora seemed practically wiped out for the time being, but a culture made after 24 hours was similar to that from the control.

EXP. 3.—Jan. 17, 1922. Normal subject.

*Procedure:* 10 per cent mercurochrome—220 was applied to the tongue continuously for ten minutes. The excess dye was then removed by irrigating with a liter of water. The results of cultures were as follows.

| *Control* | *Culture immediately after application* | *Culture 24 hrs. later* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) A very few cols. M. tetragenus | (1) Many M. tetragenus |
| (2) Many Gram-negative cocci | (2) ∞ Green streptococci | (2) A few Gram-negative cocci |
| (3) A few green streptococci | (3) Many grey streptococci | (3) ∞ Green streptococci |
| (4) A few grey streptococci | | (4) Many grey streptococci |
| | | (5) A few diphtheroids |

*Comments:* The plates made immediately after the application showed green streptococci in almost pure culture and in vastly greater numbers than in the control culture. Mercurochrome seems to inhibit all the other organisms normally found on the tongue. After 24 hours, however, the picture was similar to that of the control.

EXP. 4.—Dec. 28, 1921. Normal subject.

*Procedure:* Meroxyl, (5%), applied to the tongue for one minute.

| *Control* | *Culture immediately after application* | *Culture 24 hrs. later* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) Many M. tetragenus | (1) ∞ M. tetragenus |
| (2) A few green streptococci | (2) ∞ Green streptococci | (2) ∞ Green streptococci |
| (3) A few grey streptococci | (3) ∞ Grey streptococci | (3) Many grey streptococci |
| (4) A few hemolytic influenza bacilli | | (4) A few hemolytic influenza bacilli |

*Comment:* Culture immediately after application showed a vast increase in the non-hemolytic streptococci at the expense of the other organisms. After 24 hours no change was made out. Result similar to those in the mercurochrome experiments.

EXP. 5.—Dec. 28, 1922. Normal subject.

*Procedure:* Meroxyl (5%) was applied to the tongue for two minutes. The excess of drug was removed by irrigating with one liter of water.

| *Control* | *Culture immediately after application* | *Culture after 24 hrs.* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) Many M. tetragenus | (1) Many M. tetragenus |
| (2) A few hemolytic influenza bacilli | (2) ∞ ∞ Green streptococci | (2) ∞ Green streptococci |
| (3) ∞ Green streptococci | | (3) Many hemolytic influenza bacilli |

*Comment:* The culture made immediately after the application of "253" showed an increase in the total number of colonies, the green streptococci being increased at the expense of the other organisms. After 24 hours the picture resembled that seen in the control.

EXP. 6.—Dec. 29, 1921. Normal subject.

*Procedure:* Meroxyl (5%) was applied to the tongue for ten minutes. The excess of the drug was then removed by irrigating with one liter of water.

| *Control* | *Culture immediately after application* | *Culture after 24 hrs.* |
|---|---|---|
| (1) Many Gram-neg. cocci | (1) 1 col. staph. albus | (1) Many Gram-neg. cocci |
| (2) 3 cols. staph. aureus | (2) ∞ Non-hemolytic streptococci | (2) A few M. tetragenus |
| (3) 1 col. staph. albus | | (3) ∞ Non-hemolytic streptococci |
| (4) ∞ Non-hemolytic streptococci | | |

*Comment:* Culture after application showed a pure plate of non-hemolytic streptococci save for one colony of staph. albus. After 24 hours, a picture similar to the control was present.

EXP. 7.—Jan. 20, 1922. Normal subject.

*Procedure:* Argyrol (25%) applied to the tongue for ten minutes. The excess of the drug was removed by irrigating with one liter of water.

| *Control* | *Culture immediately after application* | *Culture after 2½ hrs.* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) 30 cols. M. tetragenus | (1) Many M. tetragenus |
| (2) A few Gram-neg. cocci | (2) ∞ Non-hemolytic streptococci | (2) ∞ Non-hemolytic streptococci |
| (3) Many non-hemolytic streptococci | (3) 50 cols. hemolytic streptococci | (3) A few Gram-neg. cocci |
| (4) A few diphtheroids | | (4) Many diphtheroids |

*Comment:* Following the application of argyrol the non-hemolytic streptococci were temporarily much increased at the expense of the remaining organisms.

EXP. 8.—Jan. 21, 1922. Normal subject.

*Procedure:* Argyrol (25%) applied to the tongue for ten minutes. The excess of the drug was removed by irrigation with one liter of water.

| *Control* | *Culture immediately after application* | *Culture after 2½ hrs.* |
|---|---|---|
| (1) Many Gram-neg. cocci | (1) ∞ ∞ Non-hemolytic streptococci | (1) A few Gram-neg. cocci |
| (2) ∞ Non-hemolytic streptococci | (2) 3 cols. diphtheroids | (2) ∞ Non-hemolytic streptococci |
| (3) A few diphtheroids | | (3) Many hemolytic influenza bacilli |
| (4) A few hemolytic influenza bacilli | | (4) A few diphtheroids |
| (5) 1 colony of a yeast | | |

EXP. 9.—Jan. 31, 1922. Normal subject.

*Procedure:* Tongue swabbed for five minutes with 1 : 10000 potassium permanganate followed by irrigation with several liters of water.

| *Control culture* | *Culture immediately after application* | *Cultures 2½ hrs. later* |
|---|---|---|
| (1) ∞ M. tetragenus | (1) 6 cols. Gram-neg. cocci | (1) ∞ M. tetragenus |
| (2) ∞ Gram-neg. cocci | (2) Several hundred grey streptococci | (2) Many Gram-neg. cocci |
| (3) Many grey streptococci | | (3) Many green and grey streptococci |
| (4) A few green streptococci | | (4) Many hemolytic influenza bacilli |
| (5) A few hemolytic influenza bacilli | | |

*Comment:* A very marked reduction in the total number of colonies followed the treatment. The effect was, however, a temporary one.

EXP. 10.—Jan. 31, 1922. Normal subject.

*Procedure:* Tongue swabbed for ten minutes with 1 : 10000 potassium permanganate. At the end of this period a very unpleasant dry astringent sensation led to a cessation of the experiment. Excess of drug removed by irrigating with water.

| *Control culture* | *Culture immediately after procedure* | *Culture 2½ hrs. later* |
|---|---|---|
| (1) Many tetragenus | (1) 6 cols. non-hemolytic streptococci | (1) Many tetragenus |
| (5) A few cols. Gram-neg. cocci | | (2) Many Gram-neg. cocci |
| (6) ∞ Non-hemolytic streptococci | | (4) ∞ Non-hemolytic streptococci |
| (7) A few diphtheroids | | |

*Comment:* A very marked immediate reduction, but no permanent change.

*Discussion of Experiments 1 to 10.*—Antiseptic drugs of various sorts for the most part in strengths adequate to inhibit bacteria in the test-tube were applied to the normal mucous membrane of the tongue to determine what modifications in bacterial flora might result. In no case was it possible completely to sterilize the experimental area, although after treatment with 1 : 10000 potassium permanganate for ten minutes only very few organisms were recovered. It seems of special interest that following the application of "220" a tremendous increase in the number of colonies of green streptococci which can be recovered in surface culture occurs at the expense of the remaining flora. Similar results are obtained with meroxyl with argyrol 25 per cent. In all the experiments it was found that the flora had reverted to "normal" within 24 hours. Whether this was due merely to the sweeping in of organisms from the surrounding areas or whether growth was reestablished *in situ* is uncertain. The latter seems more likely. In any case it is apparent that, unless the entire mouth cavity can be completely sterilized, conditions will promptly return to their previous state within a short time after the application of an antiseptic.

Attempts were made to determine the effects of applications of antiseptics on special organisms harbored in a focus of infection.

CASE I. F.—This subject, clinically well at the time, was harboring streptococcus hemolyticus in the tonsils following an acute tonsillitis.

May 27, 1921. The right tonsil was thoroughly swabbed with mercurochrome—220 (10%). Cultures were made before and after the procedure.

| *Control* | *Culture immediately after application* | *Culture 2 hrs. later* |
|---|---|---|
| (1) ∞ Beta hemolytic streptococci | (1) 5 cols. Gram-neg. cocci | (1) ∞ Beta hemol.strep |
| (2) Many Gram-neg. cocci | (2) 4 cols. non-hemol. streptococci | (2) Many Gram-neg. cocci |
| (3) 1 col. Staph. albus | | (3) 20 cols. Staph. aureus |
| (4) A few non-hemol. strep. | | (4) A few non-hemol. strep. |

*Comment:* Cultures made after application of 220 to the tonsil yielded very few colonies and no hemolytic streptococci. This was probably due to the fact that dye was carried over on the swab. Two hours later, however, although the tonsil was still deep red from the dye, more hemolytic streptococci were recovered than in the control.

May 31, 1921. Similar procedure but with the use of protargol 20%.

| *Control* | *Culture immediately after application* | *Culture 2 hrs. later* |
|---|---|---|
| (1) 200 cols. beta hemolytic strep. | (1) 100 cols. beta hemolytic strep. | (1) ∞ Beta hemolytic strep. |
| (2) Many Gram-neg. cocci | (2) Many Gram-neg. cocci | (2) Many Gram-neg. cocci |
| (3) A few non-hemolytic streptococci | (3) Many non-hemolytic streptococci | (3) Many non-hemolytic streptococci |
| (4) A few diphtheroids | (4) A few diphtheroids | (4) A few diphtheroids |

*Comment:* There was practically no alteration in flora. More hemolytic streptococci were recovered after than before the application.

CASE II. B.—This person had carried Staph. aureus in his tonsils for over a year. He was clinically well and the tonsils were small and superficially clean.

June 1, 1921. The right tonsil was swabbed with mercurochrome—220 (10%) and cultures were made from it before and after the treatment.

| *Control* | *Culture immediately after application* | *Culture 1 hr. later* |
|---|---|---|
| (1) 75 cols. Staph. aureus | (1) 3 cols. Gram-neg. cocci | (1) 50 cols. Staph. aureus |
| (2) ∞ cols. Gram-neg. cocci | (2) 100 cols. non-hemolytic strep. | (2) 6 cols. hemolytic influenza bacillus |
| (3) Many non-hemolytic streptococci | | (3) ∞ Gram - neg. cocci |
| (4) Many diphtheroids | | (4) ∞ Non - hemolytic strep. |
| | | (5) A few diphtheroids |

*Comment:* The swabbing showed an immediate reduction in cultivable organisms probably due to a carrying over of the dye on the swab. Within one hour, however, more colonies were present than in the control.

June 3, 1921. A similar experiment was done, 20% protargol being employed.

| *Control culture* | *Culture immediately after application* |
|---|---|
| (1) ∞ Gram-neg. cocci | (1) ∞ Gram-neg. cocci |
| (2) 10 cols. hemolytic influenza bacillus | (2) 5 cols. hemolytic influenza bacillus |
| (3) Many green streptococci | (3) Many green streptococci |
| (4) A few cols. diphtheroid | (4) 20 cols. Staph. aureus |
| (5) 1 colony Staph. aureus | |

*Comment:* Not only was there no total reduction in colonies but more staphylococci were recovered after the application than before.

Case III. E.—A clinically healthy man who harbored hemolytic streptococci in the tonsils.

June 6, 1921. The right tonsil was swabbed with protargol (20%) and cultures were made before and after the treatment.

| *Control* | *Culture immediately after application* | *Culture 1 hr. later* |
|---|---|---|
| (1) 20 cols. hemolytic strep. | (1) 25 cols. hemolytic strep. | (1) 75 cols. hemolytic strep. |
| (2) ∞ Cols. non-hemolytic strep. | (2) ∞ Cols. non-hemolytic strep. | (2) ∞ Cols. non-hemolytic strept. |
| (3) 10 cols. Staph. albus | (3) Many Gram-neg. cocci | (3) ∞ Gram-neg. cocci |
| (4) Many Gram-neg. cocci | | (4) 8 cols. albus |

June 9, 1921. The right tonsil was swabbed with 1 : 10000 potassium permanganate.

| *Control* | *Culture immediately after application* | *Culture 2 hrs. later* |
|---|---|---|
| (1) ∞ Green streptococci | (1) A few Gram-neg. cocci | (1) ∞ Green streptococci |
| (2) 50 cols. Gram-neg. cocci | | (2) Many Gram-neg. cocci |
| (3) 500 cols. Staph. aureus | | (3) 8 cols. Staph. aureus |
| (4) 50 cols. hemolytic strep. | | (4) 75 cols. hemolytic strept. |

*Comment:* A very marked reduction in the total number of colonies. Within two hours, however, the organisms had reappeared in proportions similar to those in the control.

Case IV. M.—A healthy woman whose tonsils had constantly yielded hemolytic influenza bacilli for over a year.

June 6, 1921. The right tonsil was swabbed with protargol (20%) and cultures were made before and after the treatment.

| *Control* | *Culture after application* | *Culture after 2 hrs.* |
|---|---|---|
| (1) 100 cols. hemolytic influenza bacillus | (1) 20 cols. hemolytic influenza bacilli | (1) Several hundred hemolytic influenza bacilli |
| (2) ∞ Green streptococci | (2) ∞ Green streptococci | (2) ∞ Green strept. |
| (3) Many Gram-neg. cocci | (3) Many Gram-neg. cocci | (3) Many Gram - neg. cocci |
| (4) 20 cols. diphtheroid | | |

Case V. H.—A healthy man with small clean tonsils. No previous examinations had been made.

June 13, 1921. The right tonsil was swabbed with mercurochrome—220 (10%) and cultures were made before and after the procedure.

| *Control* | *Culture immediately after application* | *Culture after 1 hr.* |
|---|---|---|
| (1) 20 cols. hemolytic influenza bacillus | (1) 5 cols. hemolytic influenza bacilli | (1) 30 cols. hemolytic influenza bacilli |
| (2) ∞ cols. hemolytic strept. | (2) Many cols. hemolytic streptococci | (2) ∞ Cols. hemolytic streptococci |
| (3) A few diphtheroids | (3) Many diphtheroids | (3) ∞ Gram-neg. cocci |
| (4) 20 cols. Gram-neg. cocci | (4) 100 cols. Gram-neg. cocci | (4) A few diphtheroids |
| | | (5) Many cols. Staph. albus |

## Discussion

The experiments show that while it was possible in some cases to modify the bacterial flora by intensive treatments no essential or permanent alteration could be produced. It seems pertinent, therefore, to inquire into the causes of such failures and to reconcile them with the strikingly different effects obtained *in vitro*. In a previous paper [3] it was shown that the persistence of organisms in the mouth depended upon the establishment of a vital reaction between the bacteria and the mucous membranes of the host involving a close relationship both functionally and anatomically, and that the organisms were growing not only on the mucous membrane but in the superficial layers as well—probably in crevices between the epithelial cells and in the orifices of the small mucous glands. From these niduses of growth bacteria are discharged into the mouth secretions, but the niduses themselves remain more or less intact. Under other conditions organisms may become established in a focus of diseased tissue such as tonsil, adenoid or sinus in a somewhat analogous manner. It is obvious then, that the bacteria actually multiplying are very firmly lodged in the tissues and are not growing free on the surface or in the mouth secretions. It seems unlikely, therefore, that chemicals applied to the mucous surfaces could effect sterilization without destroying the superficial layers of epithelium at the same time. In this connection the mercurochrome experiments are of special interest. This drug penetrates to such an extent that the treated membrane shows a distinct red color even after twenty-four hours. None the less the flora remains essentially unaffected.

An attempt should be made to reconcile these findings with the clinical impressions of experienced laryngologists to the effect that the application of antiseptics is of value in the treatment of acute and chronic inflammations of the nose and throat. It is possible, in the first place, that inflammatory processes due to some unknown type of virus [colds (?), pharyngitis (?), rhinitis (?)] are affected by antiseptics in a manner different from those due to known bacteria, or it may be that a beneficial effect indirectly follows irritation, hyperæmia, or ischæmia

produced by the drug. In view of these experiments, however, it seems that conservatism is necessary in interpreting clinical results.

If one regards the desiderata in treating an inflammation of the upper air passages as drainage, a wet dressing, and (in some conditions) stimulation, it would seem that a drug might be better withheld unless it definitely promotes the above conditions. It may be that in acute coryza, pharyngitis, tonsillitis, and rhinitis better results will follow the use of bland washes such as salt solution, and drugs which aid drainage by shrinking the mucous membranes such as cocaine and adrenaline, rather than the strong and irritating antiseptics, such as silver and and mercury.

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# HYPOGLYCEMIA IN EXOPHTHALMIC GOITRE

## A PRELIMINARY REPORT

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Glycemia in deranged thyroid function has been the subject of considerable study, such observations as have been made pointing to a hyperglycemia, with a diminished sugar tolerance, in cases of severe hyperthyroidism. These observations have not, however, been carried into the period immediately following operation for exophthalmic goitre.

It is generally conceded that the handling and manipulation of the gland in the course of an operation for severe thyroid intoxication results in flooding the general circulation with a considerable amount of thyroid secretion. The immediate effect of this active substance is to produce a tremendous increase in the metabolic processes of the body, necessitating a utilization of all the available carbohydrates, including the glycogen reserve in liver and muscle tissues. It is logical to suppose, therefore, that if sufficient active thyroid secretion is taken up by the circulation, there will come a moment when the available carbohydrate supply in the body is exhausted, and hypoglycemia supervenes. That this is actually the case is illustrated by the following study:

T. K., a young school girl of 17, was admitted to the Johns Hopkins Hospital on December 1, 1922, with symptoms of very marked hyperthyroidism and a diffuse enlargement of the thyroid gland. Clinically, her symptoms and appearance suggested a very severe thyroid intoxication, although her metabolic rate on December 5th was only +29 per cent with a resting pulse of 130–140.

After 14 days' rest in bed the metabolic rate had increased to +35 per cent. On December 19th the superior vessels on both sides were ligated and ten days later the metabolic rate had decreased to +29.9 per cent. There was marked subjective improvement and accordingly on January 5th, 1923, a double subtotal resection was performed.

A very severe reaction set in, as evidenced by a constantly maintained pulse of 180–200, beginning immediately after the operation and continuing throughout the next sixty hours.

Twenty-four hours after operation she was in a semi-stupor, from which she could be aroused only by considerable effort. The most striking features at this time were the tachycardia, fever of 102.4° F., extreme restlessness with evidence of very marked weakness, and shallow, rapid breathing. Her condition seemed most critical.

On the basis of the above reasoning with respect to a probable hypoglycemia, a blood sugar determination was immediately made and found to be .048 gm. per 100 cc. It is rather generally held that .09–.110 gm. per 100 cc. is a normal glycemia and that coma supervenes when .04 gm. is reached.

The rational therapy in the presence of this low blood sugar was the administration of glucose. Accordingly, 40 grammes of glucose in a 20% solution were introduced very slowly, intravenously. There was an immediate, startling improvement in the patient's condition, as evidenced by a rational response to numerous questions, and the drinking of over 500 cc. of water.

Six hours later, however, her condition again became desperate. She lay in a deep stupor from which she could not be aroused except by the greatest effort, her color had become ashen pale, the respirations were very irregular, now labored, now shallow, with a constant mucous rattle in her throat. Her pulse remained around 180. It seemed quite impossible that she would survive long. Accordingly, without waiting for another blood sugar determination, 50 gms. of glucose in a 20% solution were administered intravenously. Again there was an immediate response by an improved color, more regular respirations, but without return to complete consciousness as before. This improvement was not well sustained during the next six hours, but from this point on there was gradual betterment eventuating in complete recovery.

Ten hours after the second administration of glucose, there was a blood sugar of .119 gm. per 100 cc., and a $CO_2$ content of 45 volumes per cent. There was at no time any acetone odor to her breath, and only one specimen of urine taken during the morning following operation contained acetone and diacetic acid. Numerous other specimens during these two days were negative for acetonuria.

It seems evident that we are dealing in these cases with a hypoglycemia rather than an acidosis, and that the latter condition is probably incident to the first.

Two additional observations have been made since this illuminating case, which revealed a marked reduction in blood sugar, appearing about 24–36 hours after operation, corresponding to the postoperative period when the greatest reaction has long been known by operators to occur.

As the accompanying table indicates, there is a marked hyperglycemia immediately after operation. This corresponds to the period of most active metabolism and the greatest mobilization of the available carbohydrate. This is followed by an abrupt fall in blood sugar content, corresponding to the exhaustion of the supply. In cases reacting favorably, there is then a rise in blood sugar, indicating probably a gradual disappearance and elimination of the active thyroid secretion.

The rationale of the administration of glucose solutions intravenously is suggested by these few studies and opens a fertile field for further investigation. A definite plan of intravenous injections of glucose will need to be evolved, controlled and dependent upon successive blood sugar determinations. A weaker solution, 5 to 10 per cent in strength, administered in quantities of 300–600 cc. would probably be preferable to the 20 per cent solution.

Our findings also justify the emphasis which this clinic has placed upon the importance of a high carbohydrate and high caloric diet in the preoperative treatment of exophthalmic goitre, and of the administration of 5 per cent glucose solution per rectum in postoperative care. The administration of large quantities of fluid by infusions, by mouth, and by rectum, undoubtedly favors the elimination of the active thyroid secretion and is therefore also an important factor in reducing the severity of postoperative reaction.

BLOOD SUGAR DETERMINATIONS

| *Patient, M. F.* | | *Patient, Lucas* | |
|---|---|---|---|
| Preoperative | .097 | Preoperative | .096 |
| 1 hour post op. | .116 | 10 minutes post op. | .149 |
| 5 hours " " | .119 | 2 hours post op. | .163 |
| 24 " " " | .078 | 4 " " " | .138 |
| 36 " " " | .120 | 24 " " " | .100 |
| 48 " " " | .142 | 36 " " " | .080 |
| 72 " " " | .143 | 72 " " " | .119 |

# NOTES ON NEW BOOKS

*Endocrinology and Metabolism.* Edited by LEWELLYS F. BARKER, R. G. HOSKINS, and H. O. MOSENTHAL. (*New York and London, Appleton & Co., 1922.*) In 5 volumes.

Volumes I and II deal chiefly with endocrinology. The discussion of the clinical disorders of each of the ductless glands is preceded by a review of its anatomy and histology, physiology, and pathology, each presented by a different author. Existing knowledge of the structure and functions of the endocrine organs is thus exhaustively reviewed. The clinical descriptions of the endocrine disorders are far less satisfactory than the structural and experimental studies. A large number of disease pictures, supposedly of endocrine origin, are presented, in most cases by competent clinical observers, with the proper distinction between hypothesis and proved fact. On the other hand, as an example of the typical buncombe with which endocrinology is cursed, the reader is referred to a discussion of multiglandular syndromes in Volume II. However, very few of the clinical articles are characterized by such a wide divergence of fact and fancy as is found in the particular article referred to. A history of endocrinology by Fielding H. Garrison is one of the valuable features of Volume I.

In Volumes III and IV one finds a brilliant series of reviews of various phases of the normal and pathological processes of metabolism. Volume III contains a delightfully written history of metabolism by Graham Lusk, a review of the normal processes of energy metabolism by Murlin, on nucleic acids by Walter Jones, on fat metabolism by Bloor, and on the metabolism of the proteins and carbohydrates by A. I. Ringer. Volume IV contains excellent articles on general topics, among which the most noteworthy are on fever by DuBois, on anaphylaxis by Longcope, on acidosis by Van Slyke, on disturbances of growth by McCollum. Important articles on special topics of note are those by Woodyatt on diabetes, on gout by Pratt, and by Marriott on the pathology of metabolism in infancy and childhood.

Volume V contains the bibliography for the entire series and a general index. This arrangement seems admirable. A separate index is given at the end of each volume.

These volumes represent a compilation of an enormous literature with arrangement of the material in many permutations and combinations. They have a distinct field of usefulness. On the whole they have been very well done.

W. S. McC.

*The Practice of Medicine.* By A. A. STEVENS. Cloth, $7.50. (*1922, Philadelphia, W. B. Saunders Co.*)

As a text-book of modern medicine this single volume is destined to take its place in the front rank. The author states in his preface that he has avoided deliberately such matters as are still in the controversial stage. In spite of this, one looks in vain for the omission of data from modern research that are vital to the diagnosis and treatment of disease. The newer aspects of laboratory medicine, often ignored or superficially treated in such text-books, are handled with conservatism, sound judgment and without exaggeration. Some of the newer researches concerning diabetes are lacking, but these are probably, correctly, relegated by the author to the limbo of controversial points. One has the feeling that the book was written rather more from the medical literature than from the writer's personal experience, although all subjects are handled with the good judgment that is possessed only by sound clinicians. For a text-book of approximately a thousand pages, it would be difficult to suggest improvements in the treatment and partitioning of our sum of medical knowledge.

Sept. 21, 1922 J. T. K., Jr.

*Hyperpiesia and Hyperpiesis.*. H. BATTY SHAW, M.D., F.R.C.P. $6.50. (*London, Oxford Medical Publications, 1922.*)

From the nature of the title of this monograph one was led to expect a far more satisfactory discussion of the condition of hyperpiesia and all its attendant phenomena.

There is, to be sure, a vast amount of detailed study of forty-seven cases which the writer groups as belonging to that somewhat anomalous symptom complex characterized by hyperpiesis or hypertension. The work is essentially based upon the author's own clinical, pathological and experimental observations and it is to be regretted, it seems to us, that a review of a subject of such interest should not include a wider résumé of the literature and references to much of the more recent work.

The general arrangement of the volume is divided into four sections, the first being given up entirely to clinical and post-mortem reports of the cases referred to, given in what seems to us vastly too great detail, the first seventy-five pages in fact being devoted solely to the history and pathological reports.

In Section II these forty-seven cases are further analyzed in greater detail from the standpoint of occupation, duration of symptoms and symptomatology with particular reference to the development of their high blood pressure. This section includes cardiac findings, vascular and retinal changes and an exhaustive analysis of the clinical details of hyperpiesia with its pathology.

Section III discusses the origin of hyperpiesia and idiopathic cardiac hypertrophy. In Section IV is given the experimental evidence for the conclusion which apparently seems to the author most reasonable for the existence of pressor substances as a cause for the increase in blood pressure. It does not seem to us by any means established that the pressor substance or substances responsible for the rise in blood pressure are any more clearly established than previously. The experimental observations analyzed in Tables 6, 7 and 8 are interesting, but do not seem particularly conclusive. It is greatly to be regretted that the experimental evidence in which the author has for long been so conspicuously interested has not been developed more in detail as the really interesting part of the text. The references to the literature in connection with the pressor substances are many of them dated before 1912, which gives one the impression that this part of the work was carried out a number of years ago. The final chapter is devoted to the author's conclusions as to the nature of the underlying factors which are responsible for hyperpiesia; the soundness of his conclusions must be left as yet undetermined. The therapy is discussed in detail. The volume as a whole has disappointed us. It is diffuse, with too great attention given to unnecessary detail. It is the sort of monograph in which, if one can read it carefully, he will find expressed the author's views and impressions and theories based upon his own series of cases given in what is to us a totally uninspiring way.

E. P. C.

*A Book of Portraits of The Faculty of The Medical Department of The Johns Hopkins University, Baltimore.* By DORIS ULMANN. $20.00 net. (*The Johns Hopkins Press, Baltimore, Md., 1922.*)

This book of portraits represents an effort on the part of Miss Ulmann to portray the personality and character of the Faculty of the Medical Department of The Johns Hopkins University. The photographs were especially taken by the artist-author and the majority of them are entirely successful. The type also was designed for the volume, and the book throughout is artistic and beautiful. By its examination one who has known the Faculty of the Medical Department can call to mind vividly its former personnel. Here the original members of the group who initiated teaching in the Medical Department in 1893 are before you, with the exception of Professors Osler, Halsted and Mall, now deceased. The pictures are life-like and display a keen appreciation on the part of the author of the characteristics of the persons represented. In future years, the book will possess great value as an historical representation of the men who made the Medical School a genuine power in advancing medical education in the United States. The reproduction of the west front of the Administration Building of The Johns Hopkins Hospital which constitutes the frontispiece is an admirable work of art.

H. M. H.

*Clinical Medicine:* Tuesday Clinics at Johns Hopkins Hospital. By LEWELLYS F. BAKER, M.B., LL.D. $7.00. (*Phila. & London, W. B. Saunders Co., 1922.*)

Since the appearance of Cabot's "Differential Diagnosis" the "case history" method of presenting clinical subjects has become a popular one in American medical literature. Its ultimate value as a mode of exposition can probably not yet be fairly judged; there is an obvious appeal in the dramatic presentation of a concrete instance of disease with its special problems and lessons, which can hardly be equalled by a general textbook discussion, but on the other hand a casual incompleteness encourages, in the case of inexperienced readers, only superficial learning. To the reviewer the case history method seems to have two really useful potentialities. In the first place, it may bring to a wide and otherwise unreachable group of students the personality of an outstanding clinical teacher; secondly, it may illustrate some definite point of view in regard to the approach of clinical problems. The mere haphazard reporting, in questionable literary style, of case histories, even if they are of intrinsic interest, seems to us of little use.

In the volume under consideration one experiences a considerable degree of satisfaction. Whatever questionable there may be in the "case history" method in general, the writer has without doubt done the very best that can be achieved within the limit set. One feels that he has succeeded despite, rather than because of, his method. The first clinic of the series is introduced by a discussion of the "mental technique" of diagnosis invaluable to the undergraduate, and well worth consideration by any of us in a day burdened by "methods." As regards the clinics themselves, one can fairly say that they reproduce in a delightful and vivid way the writer's masterful presentation of medical problems in the amphitheater.

A. L. B.

*Clinical Diagnosis and Symptoms.* 2 Volumes. By ALFRED MARTINET, M.D., Paris, France, with the collaboration of Drs. Desfosses, G. Laurens, Leon Meunier, Lutier, Saint-Cène, and Terson. Authorized translation from 3rd, revised and enlarged edition.—By Louis T. deM. Sajous, B. S., M. D., $14.00. (*Philadelphia, F. A. Davis Co., 1922.*)

These two volumes should be of great value to anyone doing clinical diagnostic work. In the first the authors cover very thoroughly physical and laboratory diagnosis. All of the latest laboratory methods are given in detail, and many valuable illustrations are also presented.

In the second volume Dr. Martinet and his collaborators give an analysis of the symptoms most frequently encountered in ordinary practice. They also add a brief review of the anatomical and physiological features that are related to these symptoms. In this volume also there are many helpful illustrations. Dr. Louis T. Sajous has made an excellent translation of this important work.

J. G. H.

*Bowel Diseases in the Tropics.* By SIR LEONARD ROGERS. (*London, Henry Frowde; and Hodder and Stoughton, 1921.*)

The subjects dealt with are cholera, the dysenteries, liver abscess and sprue. Dr. Rogers has spent most of his life in the tropics working mainly upon these diseases, and from time to

time has recorded his knowledge and experiences. In this volume he has brought together his previous writings on cholera and the dysenteries, many sections of them having been rewritten in order to include the recent advances and treatment of these tropical diseases and thus bring the work up to date. There are many charts and illustrations which are very helpful in emphasizing many of the principal facts. The book is excellently written, and impresses the reader with the accurate knowledge of these diseases gained by personal observation during a long residence in the tropics. J. G. H.

*A Treatise on Glaucoma.* By ROBERT HENRY ELLIOT, M.D., B.S., (Lond.); Sc. D., (Edin.); F. R. C. S., (Eng.). Second edition. Cloth, $8.00. (*London, Henry Frowde; and Hodder and Stoughton, 1922.*)

In the present work Col. Elliot gives us pretty much all that is known today about a very difficult, not to say baffling, subject. The way in which he has dealt with a vast literature and epitomized the present day knowledge of Glaucoma is admirable; and the references placed at the end of each chapter render the sources of information readily accessible. He gives credit to others for work done, and where their views differ from his, his treatment of them is marked by a rare spirit of tolerance and equity.

His interpretation and handling of intra-ocular tension show clarity and conciseness; they mark another step forward in the solution of this perplexing question.

Operative interference for intra-ocular tension is still debatable and no fixed rule can as yet be laid down. The advisability of drug therapy and iridectomy (the La Grande or Elliot procedure) depends upon the personal opinion of the individual operator and no hard and fast line can be drawn.

The book will make interesting and instructive reading for the ophthalmologist as well as for the general practitioner. It can be heartily recommended. L. T. G.

*Reports on the St. Andrew's Institute for Clinical Research.* Vol. I. Cloth, $3.00. (*London, Henry Frowde; and Hodder and Stoughton, 1922.*)

With the development of methods in medicine which to a certain extent may be regarded as precise, there has perhaps been a tendency to neglect the study of disease from the standpoint of the natural historian. Sir James MacKenzie, a pioneer in clinical research of the modern type, has always stood out as a reactionary, and the founding of the new St. Andrew's Institute represents, perhaps, a concrete expression of his point of view. It is with interest, therefore, that one approaches the first volume of reports from the St. Andrew's Institute for Clinical Research, of which Sir James is the honorary director.

As briefly stated in the introduction, the object of his work, and that of his associates, is "the study of diseases common among the people," and a further elaboration of this idea seems to indicate that such study is to be carried out primarily along the lines of simple clinical observation. Thus, the chapter headings include such topics as the following: "Case Taking Methods;" "Prognosis;" "The Response to Effort;" "The Theory of Disturbed Reflexes in the Production of Symptoms of Disease;" "The Response of the Eye to Effort;" "A Clinical Study of Influenza" and so forth.

The reviewer is to some extent perplexed as to how to comment upon the volume in hand. The idea undoubtedly is a good one, aiming as it does to attack those problems which do not lend themselves very readily to the more highly specialized methods of approach of the modern laboratory. At the same time one has a feeling that the actual material so far brought out represents a contribution of little essential value. We find no new point of view nor any new material, but merely a stressing of simple points which are quite obvious to every intelligent practitioner who has actually been thrown in contact with sick people. The study on influenza, for example, brings out nothing which has not already been said many times, and perhaps better and more completely by others. The chapter on case-taking methods corresponds pretty closely to the schemes outlined in any textbook of diagnosis, and so on, through the volume.

In short, while many of the papers would be extremely interesting and highly creditable as lectures given to a group of students, one questions whether they are quite worth the energy and expense of bringing them into print in their present form. It seems unfair, however, to criticize what is essentially an introductory volume, and a note of reaction in present day medicine, in which methods of pseudo-precision are undoubtedly exaggerated, may eventually turn out to be a most beneficial influence. A. L. B.

*Endocrine Glands and The Sympathetic System.* By P. LEREBOULLET, P. HARVIER, H. CARRION, A. G. GUILLAUME. Translated by F. Raoul Mason with the collaboration of Daniel R. Ayres. (*Phila. and London, J. B. Lippincott Co., 1922.*)

This volume consists of a collection of monographs by French medical men, translated into English by Mason and Ayres of New York. It is not dated and from some of the more conspicuous omissions one might infer that it is not a product of the current year. One finds no mention of Kendall's thyrosin, for example, and the whole matter of basal metabolism in relation to the glands of internal secretion—a subject of such widespread interest in this country—is disposed of in a foot-note. While most of the references are naturally to the French medical literature, considerable recognition is extended to American and English authorities. German names are not abundant.

The authors make rather much of the so-called dysthyroid status, a condition which one readily recognizes on the printed page but which becomes very elusive when it is subjected to the light of careful physical and laboratory examinations. The cases of mixed hyper- and hypo-thyroidism which are described are more likely instances of one of these conditions, complicated by accidental phenomena that commonly accompany the other condition. The line between excessive and diminished thyroid activity is being drawn more sharply since the application of the basal metabolism examination. Basing their conclusions upon some experimental work of Jorne, who produced arterial degeneration and cardiac hypertrophy through intravenous administration of epinephrin into animals, and upon some pathological findings in the adrenals of hypertensives, the authors speak rather seriously of the possible existence of a relationship between atherosclerosis and hypertension and adrenal activity. "Arteriosclerosis is not always of adrenal origin," say Dr. Harvier. Our conceptions are so different in this country, that one is surprised at the dissimilarity of inferences that are drawn with regard to matters not susceptible of exact proof.

A. C. Guillaume contributes a very well worked out chapter upon the anatomy and physiology of the vegetative nervous system, which he separates, following Bichart, from the "animal" system. It impresses one as a very good summary of knowledge in this department for the student and practitioner of medicine.

The strength of the book lies in its keen clinical observations, which are undoubtedly drawn from the fund of knowledge of bedside clinicians of large experience. The discussions of symptomatology, especially of the thyroid deficiencies, are very searching. The authors have observed, with others, a relationship between hypothyroidism and certain types of chronic rheumatism, eczemas and other conditions, which are without doubt susceptible to clinical and laboratory proof. The book is well worth reading for its clinical viewpoint, if for nothing else.

J. T. K., Jr.